Study of Biodegradation of Antineoplastic Compounds using White rot fungi

Thesis submitted to Central University of Haryana
for the award of the degree of

Doctor of Philosophy
in
Biotechnology

Submitted by

Ankush

(Roll No.: 11115)

Department of Biotechnology

School of Interdiscliplinary and Applied Sciences

Central University of Haryana

Mahendergarh-123031

December, 2021

DECLARATION

This is to certify that the material embodied in the present work, entitled "**Study of biodegradation of antineoplastic compounds using white rot fungi**" is based on my original research work. It has not been submitted, in part or full, for any other diploma or degree of any University/Institution Deemed to be University and College/Institution of National Importance. References from other works have been duly cited at the relevant places.

(Signature of the Candidate with date)

Countersigned by:-

Supervisor	Co-supervisor	Co-supervisor
Prof. Bijender Singh	Dr. Kashyap Kumar Dubey	Dr. Mrinal Kanti Mandal

Head

Department of Biotechnology

Central University of Haryana,

Mahendergarh-123031

CERTIFICATE

This is to certify that thesis entitled **"Study of biodegradation of antineoplastic compounds using white rot fungi"** submitted for the degree of **Doctor of Philosophy** in the subject of Biotechnology to Central University of Haryana, Mahendergarh is a bonafide research work carried out by **Mr. Ankush** under my supervision and guidance and no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Supervisor

Prof. Bijender Singh
Department of Biotechnology,
Central University of Haryana,
Mahendergarh-123031, INDIA

CERTIFICATE

This is to certify that thesis entitled **"Study of biodegradation of antineoplastic compounds using white rot fungi"** submitted for the degree of **Doctor of Philosophy** in the subject of Biotechnology to Central University of Haryana, Mahendergarh is a bonafide research work carried out by **Mr. Ankush** under my *supervision (till July 2021) afterward co-supervision and guidance and no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Co-supervisor

Dr. Kashyap Kumar Dubey
School of Biotechnology,
Jawaharlal Nehru University,
New Delhi-110067, INDIA

Immediate past affiliation
Department of Biotechnology,
Central University of Haryana,
Jant-Pali, Mahendergarh-123031, INDIA

CERTIFICATE

This is to certify that thesis entitled **"Study of biodegradation of antineoplastic compounds using white rot fungi"** submitted for the degree of **Doctor of Philosophy** in the subject of Biotechnology to Central University of Haryana, Mahendergarh is a bonafide research work carried out by **Mr. Ankush** under my co-supervision and guidance and no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Co-supervisor

Dr. Mrinal Kanti Mandal
Associate Professor
Department of Chemical Engineering,
National Institute of Technology,
Durgapur-713209, INDIA

CERTIFICATE OF ORIGINALITY

This is to certify that the research work embodied in this thesis, entitled "**Study of biodegradation of antineoplastic compounds using white rot fungi**" submitted for consideration for the award of Doctorate of Philosophy in Biotechnology is an original work carried out by me at the Department of Biotechnology, Central University of Haryana, Mahendergarh, India. The manuscript has been subjected to plagiarism check by Turnitin software.

(Signature of the Candidate with date)

ACKNOWLEDGEMENTS

At first, I wish to thank the almighty for giving me powers to complete my entire course and research program. I wish to deliver my sincere thanks to Dr. Kashyap Kumar Dubey, School of Biotechnology, Jawaharlal Nehru University, New Delhi, whose philosophical and practical advice always helped me to follow this tough path with ease. He always listened to me and provided everything in terms of encouragement, support, boost and a familiar environment in the lab. I will never forget his continuous moral support, encouraging attitude, critical analysis, judgments, valuable suggestions and friendly behaviour that has been my great source of strength throughout my research work. I will be always obliged for him for providing me opportunity to work as research scholar under his kind guidance, listening to my research ideas with patience and treating me as friend not like a student during this whole journey. Sir, thank you very much.

I would like to convey my sincere thanks to Head of the Department Biotechnology, Prof. Bijender Singh for providing research facility, encouragement and support during the tenure of this study.

I am also thankful to faculty member of Biotechnology Department, Prof. Dinesh Kumar, Dr. Ram gopal Nitharwal, Dr. Ravi Kumar, Dr. Namrata Dhaka and Dr. Inderjeet Kaur for their constant help and motivation.

I would like to acknowledge Dr. Mrinal Kanti Mandal, Dr. Vinod Kumar, Dr. Gulab Singh, Prof. Satish Kumar, Dr. Ashwani, Dr. Pawan Kumar Maurya, Dr. Avijit Parmanik, Dr. Tejpal Dhewa and Dr. Puneet Kumar for their encouragement and help during the tenure of this study.

It gives me immense pleasure to mention the names of my lab mates Ms. Khushboo and Mrs. Manisha Sharma, Mr. Vinod Kumar, Mrs. Karuna Yadav, Mrs. Pinky, Ms. Indu and Mrs. Pratiksha for their constant help and support during this journey.

No words can adequately convey my thanks to my friends, batch mates, my seniors and my junior colleagues Mr. Mony Thakur, Mr. Rajkamal Vibhuti, Ms. Jyoti Yadav, Mr. Prakash Yadav, Mr. Adrash Shukla, Mr. Aman Kumar, Mr. Vikram Poria, Mr. Anand Yadav,

Ms. Himanshu, Mr. Akshay Kumar and Mr. Manjeet for their cooperation and pleasant companionship in this journey.

The endless love, affection, sacrifice and constant inspiration from my family members and relatives enabled me to touch this ambition. My vocabulary utterly fails in expressing my respect and love to whole family members for their support during study.

A big thanks to each and every person who has somewhere somehow, been linked to me during completion of my research work.

I also acknowledge the UGC, New Delhi, India for the fellowship through a CSIR-UGC-JRF during the course of this investigation. DBT, Ministry of Science and Technology, GoI, New Delhi, for Inno-Indigo research project [Grant number: BT/IN/INNO-INDIGO/26/MKM/2015-16] provided sophisticated instrument for analysis and chemicals for experimentation.

Last but certainly not the least; I am again extremely thankful to the Almighty God for his grace and blessings, which provided me courage to sail through this journey.

(Ankush)

ABSTRACT

Antineoplastic drugs released into the aquatic environment exhibit a potential threat to normal aquatic life. In present investigation, the biodegradation of three antineoplastic compounds (cyclophosphamide, etoposide and paclitaxel) by white rot fungi *Ganoderma lucidum* (MTCC-1039), *Trametes versicolor* (AH05) and *Phanerochaete chrysosporium* (MTCC-787) were explored. HPLC method development for the detection of these antineoplastic compounds and cytotoxicity on Raw 264.7 cell line was also investigated. Three simple, sensitive, robust and accurate method developed for the detection and quantification of selected antineoplastic compounds using HPLC and validated the same. The retention time for cyclophosphamide, etoposide and paclitaxel were 3:32.3 ±0.41, 2:40.1 ±0.48 and 3:49.3 ±0.28 minutes respectively. In biodegradation, the highest biodegradation rate was achieved in *G. lucidum* mediated treatment. Herein, the biodegradation efficiency for cyclophosphamide, etoposide and paclitaxel was 98.49 %, 71.50 % and 19.54 % respectively achieved. Whereas the total removal efficiency including sorption of these compounds with *G. lucidum* was 75.56, 99.69 and 21.84 % respectively. The degradation kinetics study followed pseudo-first-order reaction for the analysis. The maximum rate constant shown in *G. lucidum* mediated treatment, where $t_{1/2}$ for initial concentration of cyclophosphamide, etoposide and paclitaxel were calculated as 4.43189, 1.27113 and 24.31 days with rate constant (k) 0.5453 day^{-1}, 0.1564 day^{-1} and 0.0285 day^{-1} respectively. The inhibitory concentration (IC_{50}) of native form of cyclophosphamide, etoposide, and paclitaxel was determined by the in-vitro cytotoxicity analysis. The cell cytotoxicity of cyclophosphamide, etoposide paclitaxel on Raw 264.7 cells was analysed as 69.58 %, 92.01 % and 88.85 % respectively at concentration 250 $\mu g.ml^{-1}$. In *G. lucidum* mediated treatment, the maximum cell cytotoxicity of transformed products (TPs) of cyclophosphamide, etoposide and paclitaxel was analysed as 2.41 %, 1.57 % and 10.31 %

respectively. In *T. versicolor* and *P. chrysosporium* only etoposide was effectively degraded and the maximum toxicity of their TPs analysed as 2.74 and 8.03 % respectively.

Keywords: Antineoplastic compounds; degradation; TPs; biosorption; kinetics; toxicity

सार

जलीय पर्यावरण में छोड़े गए एंटीनियोप्लास्टिक दवाएं सामान्य जलीय जीवन के लिए संभावित खतरे को प्रदर्शित करती हैं। वर्तमान जांच में, सफेद सड़ांध कवक गैनोडर्मा ल्यूसिडम (एमटीसीसी-1039), ट्रैमेट्स वर्सिकोलर (एएच05) और फेनेरोकीट क्राइसोस्पोरियम (एमटीसीसी-787) द्वारा तीन एंटीनियोप्लास्टिक यौगिकों (साइक्लोफॉस्फॉमाइड, एटोपोसाइड और पैक्लिटैक्सेल) के जैव निम्नीकरण का पता लगाया गया था। इन एंटीनियोप्लास्टिक यौगिकों की विषाक्तता का पता लगाने के लिए रॉ 264.7 कोशिका लाइन तथा जलीय पर्यावरण में पता लगाने और मात्रा के लिए एचपीएलसी विधि विकास की भी जांच की गई। एचपीएलसी का उपयोग करके चयनित एंटीनियोप्लास्टिक यौगिकों का पता लगाने और मात्रा के अनुमान के लिए सरल, संवेदनशील, मजबूत और सटीक विधियां विकसित की गई और उन्हीं को मान्य किया गया। साइक्लोफॉस्फेमाइड, एटोपोसाइड और पैक्लिटैक्सेल के लिए प्रतिधारण समय क्रमशः 3:32.3 ±0.41, 2:40.1 ±0.48 और 3:49.3 ±0.28 मिनट था। जैव निम्नीकरण में, गैनोडर्मा ल्यूसिडम मध्यस्थता उपचार में उच्चतम जैव निम्नीकरण दर हासिल की गई थी। इसमें, साइक्लोफॉस्फॉमाइड, एटोपोसाइड और पैक्लिटैक्सेल के लिए जैव निम्नीकरण दक्षता क्रमशः 98.49 %, 71.50 % और 19.54 % प्राप्त की गई थी। जबकि, गैनोडर्मा ल्यूसिडम के साथ इन यौगिकों के जैव-अवशोषण सहित कुल निष्कासन दक्षता क्रमशः 75.56 %, 99.69 % और 21.84 % थी। गिरावट गतिकी अध्ययन ने विश्लेषण के लिए छद्म-प्रथम-क्रम प्रतिक्रिया का पालन किया। गैनोडर्मा ल्यूसिडम मध्यस्थता उपचार में अधिकतम दर स्थिरांक प्राप्त हुआ, जहां साइक्लोफॉस्फॉमाइड, एटोपोसाइड और पैक्लिटैक्सेल की प्रारंभिक मात्रा के लिए हाफ लाइफ ($टी_{1/2}$) की गणना 4.43189, 1.27113 और 24.31 दिन के साथ-2 दर स्थिर (के) क्रमशः 0.5453, 0.1564 और 0.0285 प्रति दिन थी। कृत्रिम परिवेशीय विषाक्तता विश्लेषण द्वारा साइक्लोफॉस्फेमाइड, एटोपोसाइड और पैक्लिटैक्सेल के मूल रूप का निरोधात्मक मात्रा ($आईसी_{50}$) निर्धारित किया गया था। रॉ 264.7 कोशिकाओं पर साइक्लोफॉस्फॉमाइड, एटोपोसाइड और पैक्लिटैक्सेल की कोशिका विषाक्तता का विश्लेषण 250 माइक्रोग्राम प्रति मिलीलीटर की मात्रा पर क्रमशः 69.58 %, 92.01 % और 88.85 % के रूप में प्राप्त हुआ था। गैनोडर्मा ल्यूसिडम मध्यस्थता उपचार में, साइक्लोफॉस्फॉमाइड, एटोपोसाइड और पैक्लिटैक्सेल के रूपांतरित उत्पादों की अधिकतम कोशिका विषाक्तता का विश्लेषण क्रमशः 2.41 %, 1.57 % और 10.31% के रूप में प्राप्त हुआ था। ट्रैमेट्स वर्सिकोलर और फेनेरोकीट क्राइसोस्पोरियम में केवल एटोपोसाइड को प्रभावी रूप से अवक्रमित किया गया था और उनके रूपांतरित उत्पाद की अधिकतम विषाक्तता का विश्लेषण क्रमशः 2.74 % और 8.03% के रूप में प्राप्त हुआ था।

संकेत-शब्द: एंटीनियोप्लास्टिक यौगिक; निम्नीकरण; रूपांतरित उत्पाद; जैव-अवशोषण; गतिकी; विषाक्तता

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Detail
STPs	Sewage treatment plants
WWTPs	Wastewater treatment plants
SPE	Solid phase extraction
HPLC	High performance liquid chromatography
SPE-GC-MS	Solid phase extraction gas chromatography mass spectrometry
SPE-LC-MS/MS	Solid phase extraction liquid chromatography tandem mass spectrometry
SPE-HPLC-ESI-MS/MS	Solid phase extraction high performance liquid chromatography electrospray ionization tandem mass spectrometry
HPLC-QqQ-MS	High performance liquid chromatography triple quadrupole mass spectrometry
LC-Orbitrap-MS	Liquid chromatography high resolution mass spectrometry
LOQ	Limit of quantification
LOD	Limit of detection
AOPs	advanced oxidation processes
HRT	Hydraulic retention time
SRT	Sludge retention time
HRL	Hydraulic load
UV-VIS	Ultraviolet visible

pK_a	Acid dissociation constant
K_{ow}	Octanol-water partition coefficient
K_{oc}	Carbon-water partition coefficient
kDa	Kilo Dalton
BCF	Bioconcentration factor
DNA	Deoxy-ribonucleic acid
RNA	Ribonucleic acid
5-FU	5-flurouracil
IF	Ifosfamide
IARC	International agency for research on cancer
GCO	Global cancer observatory
dfdU	2', 2'-difluorodeoxyuridine
Blq	Below limit of quantification
LOEC	Lowest observed effect concentration
EC_{50}	Effective concentration
LD_{50}	Lethal dose
IC_{50}	Inhibitory concentration
FBAL	α-fluoro-β-alanine
AraU	Uracil-1-β-D-arabinofuranoside
AT	Acute toxicity
CT	Chronic toxicity
PLHC	Poeciliopsis lucida hepatocytes
FASS	Federation of Animal Science Societies
ZFL	Zebrafish liver
RTG	Rainbow trout gonad

RO	Reverse osmosis
NF	Nanofiltration
Eq^n	Equation
PAC	Powder activated carbon
GAC	Granulated activated carbon
MBR	Membrane bioreactor
DOC	Dissolved organic carbon
WWPF	Wastewater and permeate flow
ORP	Oxygen and reduction potential
WRF	White-rot-fungi
CYP_{450}	Cytochrome P_{450}
R^2	Regression coefficient
ABTS	2,2'-azino-bis (3 ethylbenzthiazoline)-6-sulfonate
RPM	Rotation per minute
LB	Luria broth
OD	Optical density
PBS	Phosphate buffer saline
DMEM	Dulbecco modified eagle medium
DNSA	Dinitro salicylic acid
RT	Retention time
TPs	Transformed products

LIST OF SYMBOLS

Symbol	Detail
ºC	Degree centigrade
%	Percentage
±	Plus-minus
ƛ	Wavelength
:	Colon
(	Round bracket
[	Square bracket
–	Minus
×	Multiply
~	Tilde
/	Slash
+	Plus
;	Semicolon
=	Equal sign
µ	Mu
,	Comma
<	Less-than sign
.	Dot
>	Greater-than sign
i.e.	That is
min.	Minutes
$g.L^{-1}$	Gram per liter

$mg.L^{-1}$	Milligram per liter
$\mu g.L^{-1}$	Microgram per liter
$\mu g.ml^{-1}$	Microgram per milliliter
$ng.L^{-1}$	Nanogram per liter
PPM	Parts per million
pH	Power of hydrogen ion concentration

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).

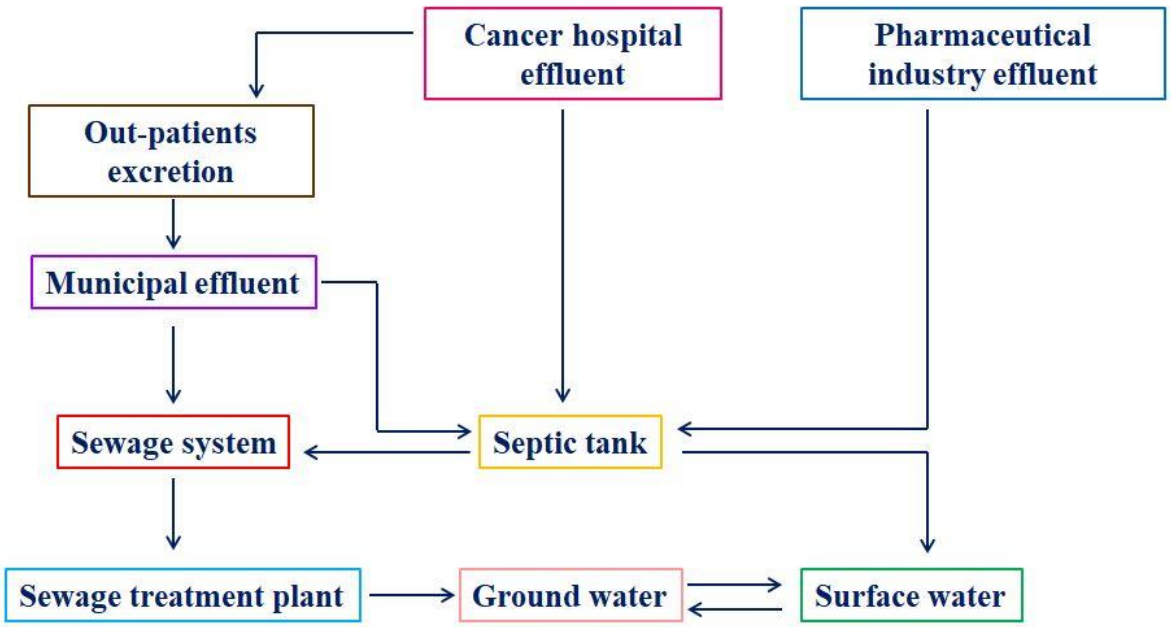


Fig. 1.1. Sources and pathway of antineoplastic drugs in aquatic environment

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

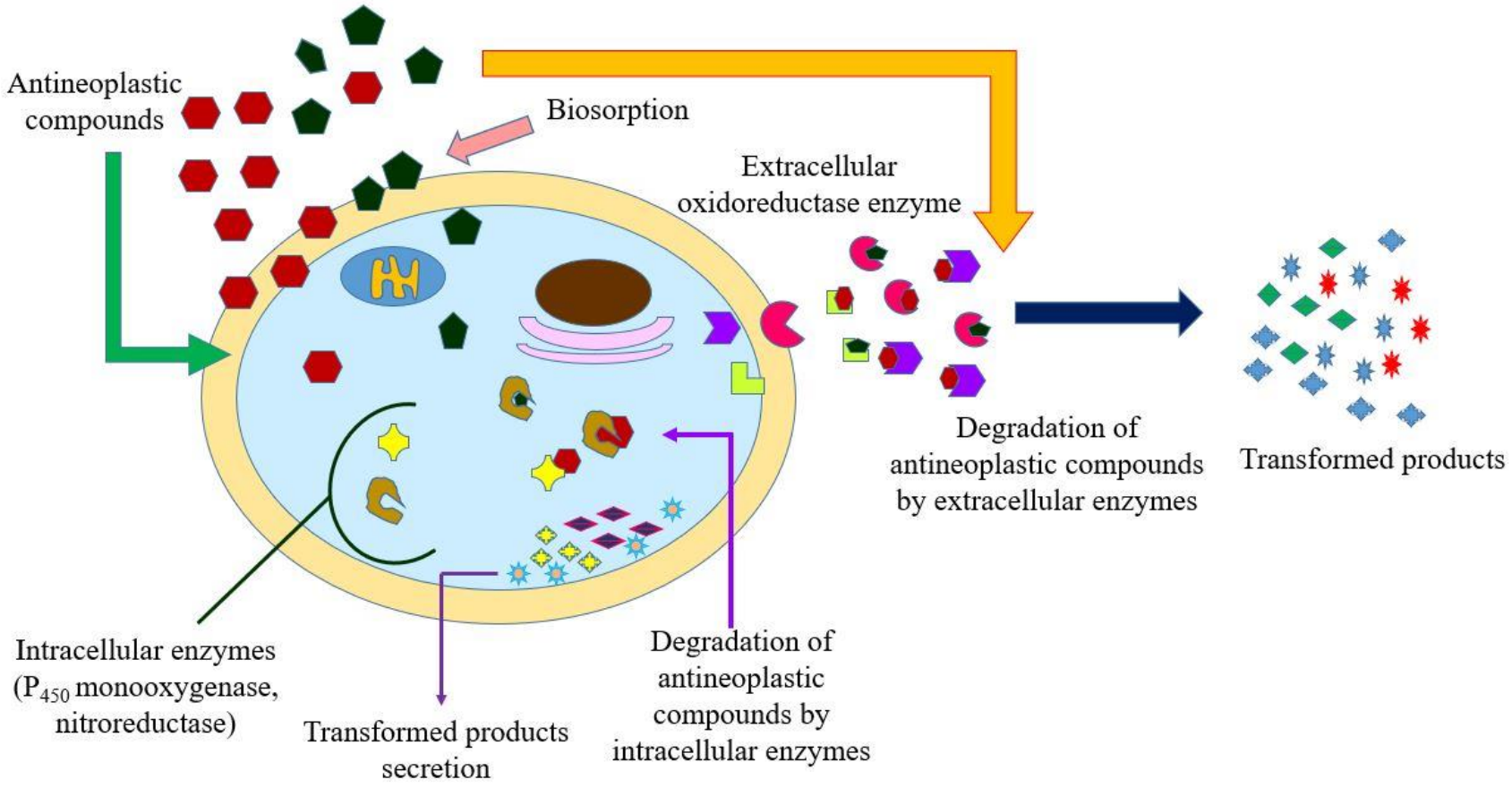


Fig. 1.2. Possible mechanism of degradation of antineoplastic compounds by white rot fungi

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).

Review of Literature

2.1. Antineoplastic compounds

In present, cancer is the 2nd leading disease at world level. The population of new cancer incidence increasing at very high rate every year. Antineoplastic compounds are a class of pharmaceutical compounds which are used as a chemotherapy agent for the treatment of neoplastic or cancer disease. These are organic compounds that inhibit the proliferation in rapidly dividing cells or abnormal growth of any cell in tissue that potentially acts like a cancer cell. The classification of cytostatic drugs is mainly based on their structure or chemical activity during cell cycle which may be either phase-specific or non-specific. In general, cytostatic compounds are classified in two major group *i.e.* antineoplastic and endocrine (hormone) therapy compounds, while antineoplastic compounds are classified mainly in five groups on the basis of their mode of action or their chemical origin *i.e.* (i) L01A- alkylating agents (ii) L01B- antimetabolites (iii) L01C- plant alkaloids) (iv) L01D- cytotoxic/antitumor antibiotics (v) L01X- other antineoplastic compounds and L02- endocrine therapy compounds (L02A and L02B) as indicating in Fig. 2.1 (Nassour et al., 2019; Toolaram et al., 2014).

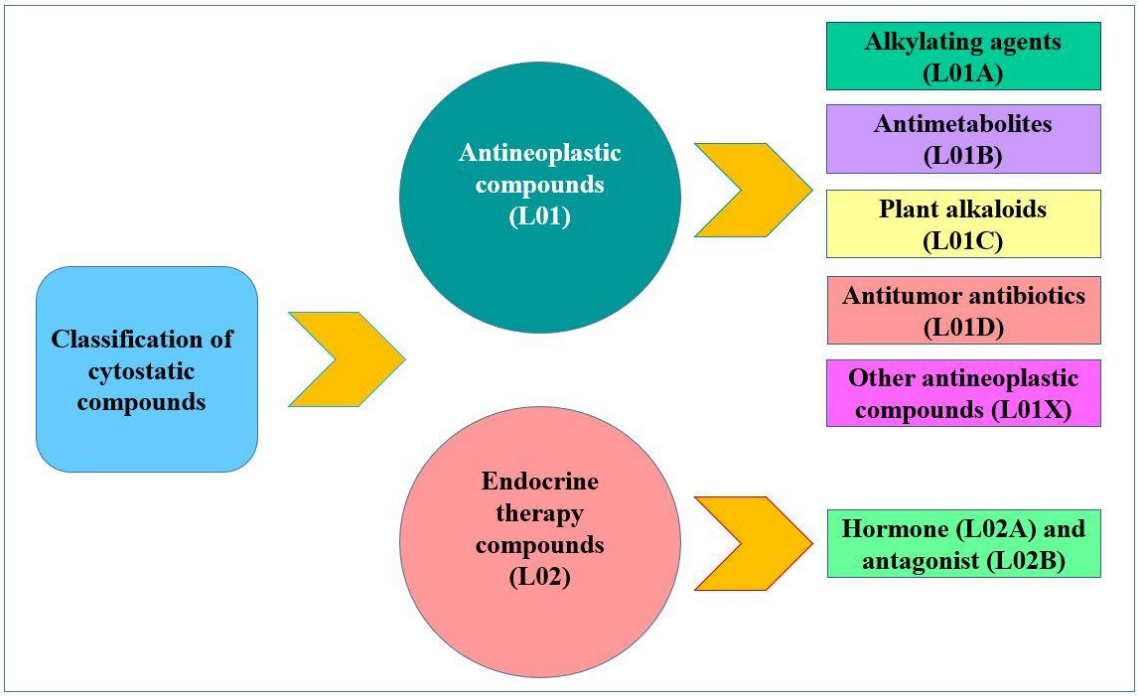


Fig. 2.1. Classification of antineoplastic agents

2.1.1. Alkylating agents (L01A)

Alkylating agents are responsible for inhibition of DNA replication during cell division. These form intra or inter cross linking with DNA strands, mismatch base pairing and strand breaking when bind with DNA strand. Alkylating agents are further divided into six sub-groups based on their chemical groups *i.e.* (1) Nitrogen mustards, includes compounds such as cyclophosphamide, chlorambucil, ifosfamide (IF), melphalan, trofosfamide and mechlorethamine. (2) Nitrosoureas, includes compounds such as carmustine, stramustine, lomustine, streptozocin. (3) Alkyl sulfonates, includes compound such as busulfan. (4) Triazines, includes compounds such as dacarbazine, procarbazine and temozolomide. (5) Ethylenimines, includes compounds such as altretamine and thiotepa. (6) Platinum drugs, includes compounds such as cisplatin, carboplatin and oxaliplatin (Ferrando Climent, 2016). These agents are commonly used in oncology wards for the treatment of leukaemia and lymphoma patients.

2.1.2. Antimetabolites (L01B)

Antimetabolites are false metabolites and interfere with RNA and DNA synthesis during cell division. They indicate similarity in structure with pyrimidine bases and block DNA replication during S-phase of cell cycle. These agents are divided into four sub-groups *i.e.* (1) Pyrimidine antagonist, includes compounds such as cytarabine, tegafur, floxuridine, azatadine, 5-flurouracil (5-FU), ftorafur (tegafur/uracil) and gemcitabine. (2) Purine antagonists, includes compounds such as thioguanine, azathioprine, mercatopurine and cladribine. (3) Adenosine antagonists, includes compounds such as fludarabine and pentostatine. (4) Folic acid antagonist, includes compounds such as methotrexate, trimetrexate and raltitrexed (Ferrando Climent, 2016). Antimetabolites shows specificity towards somatic cell division for their mode

of action. They are commonly used for treatment of leukaemia, lymphoma, pancreatic and colorectal cancer patients.

2.1.3. Plant alkaloids (L01C)

Plant alkaloids are responsible for mitotic arrest and blocking of the cell division. These compounds act by binding with microtubules protein (tubulin – globular protein) in metaphase of cell cycle. They block the assembly of mitotic spindle with kinetochore and stop cell cycle. Plant alkaloids are divided into two sub-groups which are (1) Topoisomerase inhibitor (2) Mitotic inhibitors. The topoisomerase inhibitor further divided into two sub-groups which are (i) Camptothecin, includes compound such as topotecan and irinotecan. (ii) Podophyllotoxin, includes compounds such as etoposide and teniposide. Mitotic inhibitor also divided into two sub-groups which are (i) Taxanes, includes compounds such as paclitaxel and docetaxel. (ii) Vinca alkaloid, includes compounds such as vinblastine, vincristine and vinorelbine (iii) Colchicine derivatives (Ferrando Climent, 2016; Dubey et al., 2017). Plant alkaloids are used for treatment of lymphoma, ovarian and lung cancer patients.

2.1.4. Antitumour antibiotics (L01D)

Antitumour antibiotics are not specific to cell cycle for mode of action and interference with DNA/RNA (ribonucleic acid). They can block the topoisomerase activity and base pair binding to stop the DNA replication. Antitumour antibiotics are divided into two sub-groups which are (1) Anthracyclines, includes compounds such as daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, pirarubicin and amsacrine. (2) Other antibiotics, includes compounds such as actinomycin-D, bleomycin, mitomycin-C and ciprofloxacin (Ferrando Climent, 2016). These cytostatic antibiotics are used for the treatment of acute leukaemia by induction therapy and lymphoma by combination therapy. Antitumor antibiotics are also highly effective chemotherapeutic treatment for solid tumour.

2.1.4. Other antineoplastic compounds (L01X)

This group of antineoplastic drugs act as like alkylating agent but due to their square planner form, they do not interact through electrophile alkyl group. These agents can block DNA activity by induction of inter-strand cross-linking to disrupt transcription when bind with DNA. These compounds divided in sub-groups which are (1) Platinum compounds includes carboplatin, cisplatin, oxaliplatin (2) Methylhydrazine compounds etc. These drugs are used for the treatment of ovarian and lung cancer patients by inhibition of cell division (Dehghanpour et al., 2020; Mukherjee et al., 2020).

2.1.5. Hormones and antagonists (L02)

Hormone and antagonists are responsible to block the cell cycle by changing the internal and external environment in the cell. These compounds are non-specific for any phase of cell cycle. Hormone and antagonist are divided into five sub-groups which are (1) Anti-estrogens, includes compounds such as fulvestran, tamoxifen and toremifene. (2) Aromatase inhibitors includes compounds such as anastrozole, exemestane and letrozole. (3) Progestins, includes compounds such as megestrol acetate. (4) Anti-androgens, includes compounds such as bicalutamide, flutamide and nilutamde. (5) Gonadotropin-releasing hormone, includes compounds such as leuprolide and goserelin (Ferrando Climent, 2016). These are using in oncological hospitals for the suppression or treatment of prostate, breast and ovarian cancer, so they mainly act on estrogen and androgen responsive cancers.

2.2. Antineoplastic occurrence in the aquatic environment

The worldwide average of 18.07 million new cases and about 36 new types of cancers were reported in the year 2018 (Bray et al., 2018) (Fig. 2.2) and it is expected to increase up to 29.5 million in the year 2040 (IARC, WHO [GCO]. Therefore, the use of antineoplastic compounds

will also continue to increase in the coming years and subsequently, the risk of water pollution due to antineoplastic contaminants will also continue to increase. Chemotherapy is a slow, but reliable treatment technique to treat cancer cells even after surgery or radiation therapy to remove any leftover cancer cells. The level of antineoplastic compounds reported in different environmental samples in concentration range 0.1 to 86200 $ng.L^{-1}$. Azuma, (2018) described occurrence of bicalutamide and a total of six drugs were detected in the Yodo river of Japan, out of which the highest concentration was found to be 254 $ng.L^{-1}$. The domestic wastewater discharged from hospitals usually makes their way to the sewage treatment plants and further to the water bodies. In many Asian countries, the hospitals are not well equipped with adequate WWTPs to treat/remove these pollutants. In conventional STPs, different treatment trains combining physicochemical and biological processes are used to treat wastewater. These methods only partially degrade antineoplastic compounds, while a majority of the non-treated pollutant is discharged to water bodies.

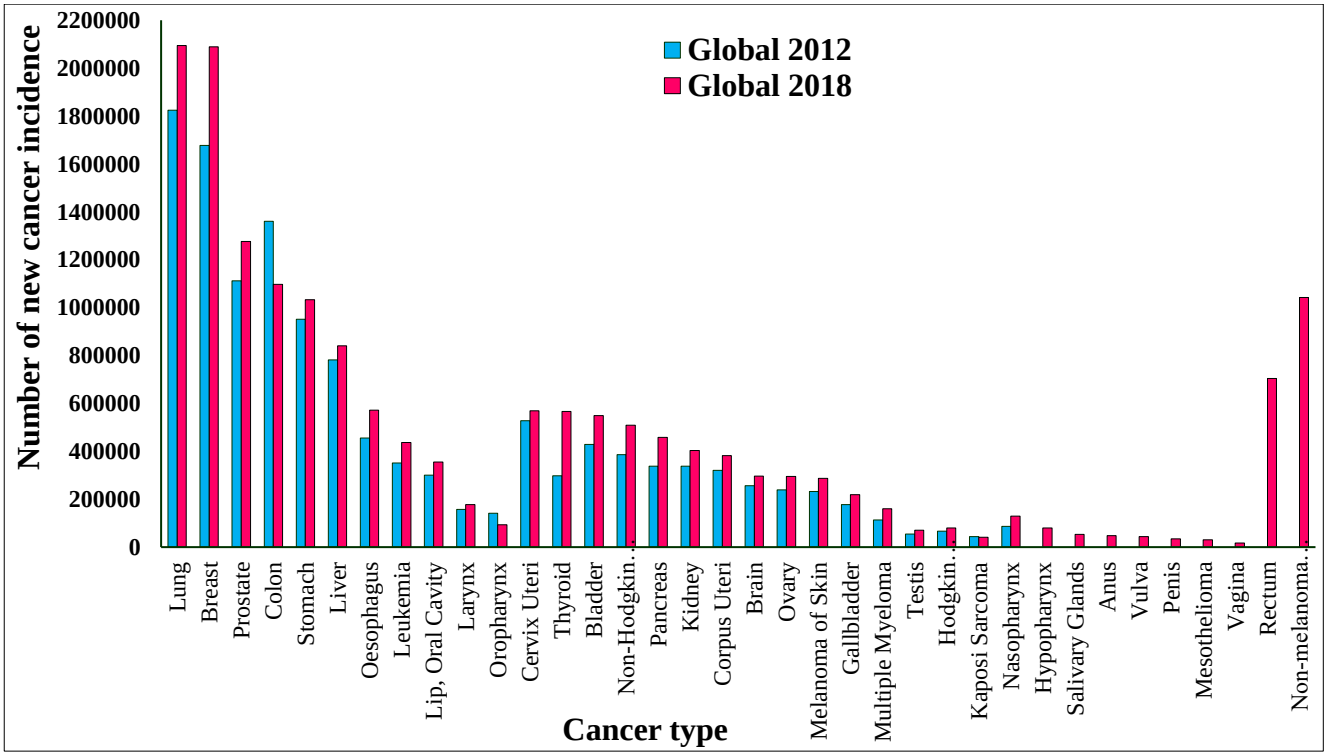


Fig. 2.2. Comparison of worldwide new cancer incidence of year 2012 and 2018

After the release of antineoplastic drugs into water bodies, both the parent compounds and their derivatives may further undergo physical and chemical interactions in water through the process of hydrolysis, photolysis, dilution, adsorption, chemical/biological accumulation, etc. among others. Due to the very low vapour pressures, most of these antineoplastic drugs are present in the liquid or solid forms in activated sludge or suspended solids. The low value of octanol-water partition coefficient (K_{ow}=10^{-3}) suggests that the compound will have low adsorption onto the solids present in water. Besides, the high value of carbon-water partition coefficient (K_{oc}) provides information about the compounds mobility in solids in comparison to water. The value of K_{ow} and K_{oc} for each antineoplastic compound varies significantly, for example, the K_{ow} value of cyclophosphamide is 0.630 and IF is 0.860 (Table 2.1). The antineoplastic drugs will also be degraded by photolysis, while high values of bioconcentration factor (BCF) indicate their accumulation in organic matter. Based on the physiochemical properties of a particular antineoplastic drug, they are present in different water environments (e.g. surface water, groundwater, rivers, lakes, oceans), at varying concentrations, including WWTPs (Table 2.2). Their degradation profiles should be monitored periodically in aquatic environments. These compounds are not degraded easily and have a long half-life; besides, their metabolites can pass from one food chain to another through aquatic species such as fish and seafood (El-Kady and Abdel-Wahhab, 2018) (Fig. 2.3).

There are various ways in which antineoplastic drugs multiply in the environment (Besse et al., 2012; Habibzadeh et al., 2018). Low vapour pressure of antineoplastic compounds leads to non-volatile nature under normal conditions; this property increases the solubility of antineoplastic drugs in water. The excretion product of the patient may be one of the reasons for the dispersion of the drugs in the surroundings because of the high solubility of antineoplastic drugs (Pruijn and De-Witte, 2004). It is noteworthy to mention that, depending on the mode of excretion, patient's gender and improper management practices during urine

accession can simply disperse the antineoplastic drugs in the environment. Nevertheless, anticancer drugs are excreted in a stable form along with urine (Polovich and Martin, 2011; Santana-Viera et al., 2016). The results of a recent contamination survey done at a hospital indicated the presence of anticancer drugs on the toilet seat and floor (Nakano et al., 2013; Sato et al., 2014). Many other surveys have also mentioned that hospital staffs and family members have more chances to be exposed to these drugs via unanticipated contact with the patient's body fluids. Besides, there are numerous pathways for antineoplastic drugs to reach the nearby water environments (Fig. 2.3). As discussed previously, hospitals are the main source of antineoplastic drugs pollution in the aquatic environment. It also depends on the persistency of the parent compound, its physiochemical property and administration mode. The consumption rate of antineoplastic drugs varies from country to country every year according to the population of cancer patients.

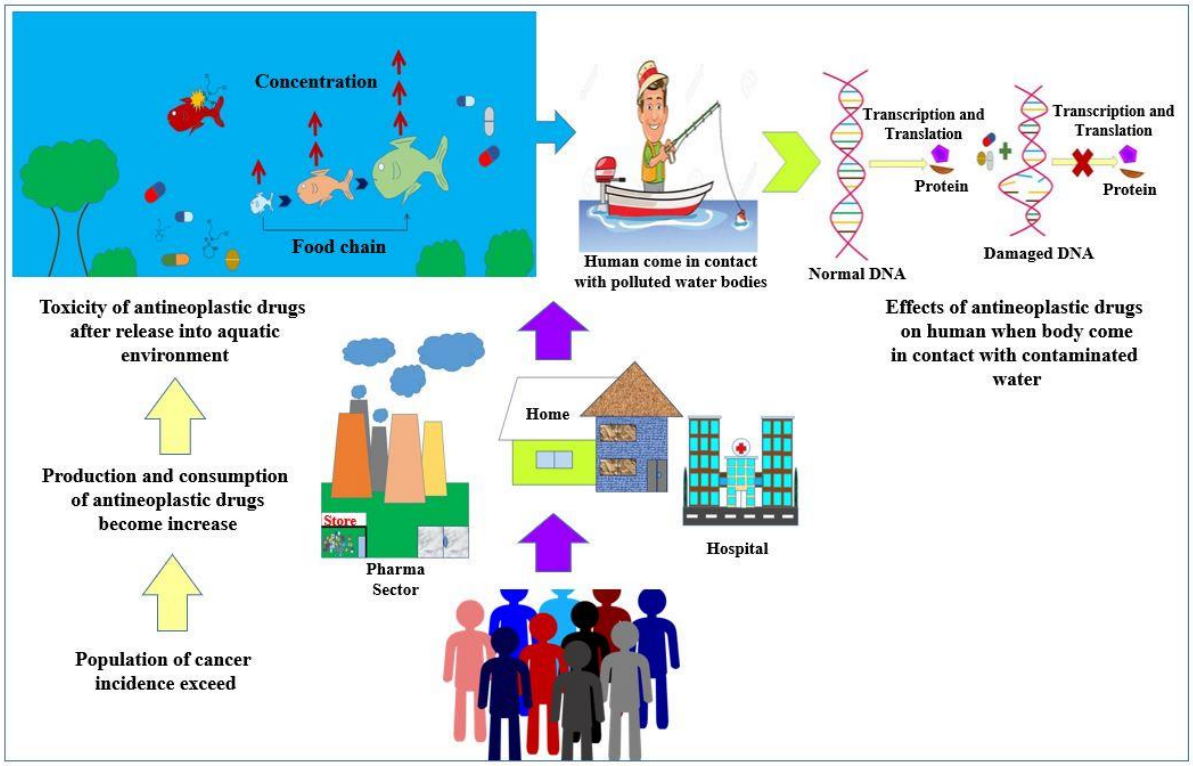


Fig. 2.3. Possible life cycle and negative effect of antineoplastic compounds after release into environment

Table 2.1. Physiochemical properties of some commonly used antineoplastic drugs

Name of the Compounds	**Applications**	**Chemical class**	**Molecular weight (g.mol^{-1})**	***pK$_a$***	**Solubility (mg.ml^{-1} in water)**	**Log *K$_{ow}$***	***Log K$_{oc}$***	**BCF**	**UV$_{max}$ (nm)**
Cyclophosphamide	• Lymphomas • Brain cancer • Neuroblastoma • Leukaemia • Some solid tumours	Alkylating agent	261.08	2.84	40	0.63	59	2.1	200
Ifosfamide	• Testicular cancer • Breast cancer • Lymphoma (Hodgkin and Non-Hodgkin) • Soft tissue sarcoma • Osteosarcoma or bone tumour	Alkylating agent	261.08	1.45	3.8	0.86	62	2.2	<290

	• Lung cancer • Cervical cancer • Ovarian cancer								
Etoposide	• Lung cancer • Testicular cancer • Lymphoma • Nonlymphocytic leukaemia • Glioblastoma multiforme	Plant alkaloid	588.57	9.8	Insoluble	0.60	51	3	283/229
Paclitaxel	• Ovarian • Breast and lung • Bladder • Prostate • Melanoma • Oesophageal	Plant alkaloid	853.93	11.9 9	Insoluble	3.95	335 5	591	227/ 273

	• Other types of solid tumour cancers								
5- Fluorouracil	• Anal cancers • Breast cancers • Colorectal cancers • Oesophageal cancers • Pancreatic cancers • Skin cancers	Antimetabolite	130.08	8.02	11.1	-0.89	8	3	266
Tamoxifen	• Breast cancer	Hormonal antagonist	371.515	8.87	0.167	7.88	DNF	827	205
Methotrexate	• Uterus cancer • Lung cancer • Breast cancer • Leukaemia • Head and Neck cancers	Antimetabolite	454.44	4.7	>1	-1.85	1	3.2	244/290

	• Lymphoma								
Capecitabine	• Colon or rectal cancer • Metastatic breast cancer • Ovarian cancer • Fallopian tube cancer	Antimetabolite	359.354	1.9	26	0.6	4.5-8.0	1.3-3.0	310
Gemcitabine	• Lung cancer • Pancreatic cancer • Bladder cancer	Antimetabolie	263.198	3.6	.546	-2.01	DNF	DNF	232
Vincristine	• Thyroid cancer • Brain tumour • Acute leukaemia • Multiple myeloma	Plant alkaloid	824.958	11.99	.0227	2.82	DNF	DNF	252/293/218/285
Doxorubicin	• Lung cancer • Breast cancer • Leukaemia	Antitumour antibiotic	543.52	8.22	26	1.27	6000	0.5	290

	• Ovarian cancer								
	• Stomach cancer								
Docetaxel	• Breast cancer	Plant	807.90	12.0	.00274	2.83	1.9	19	283
	• Head and Neck cancer	alkaloid		2			×		
	• Stomach cancer						10^6		
	• Prostate cancer								
	• Lung cancer								

Note: pK_a (Acid dissociation constant), BCF (Bioconcentration factor), DNF (Data not found)

A study conducted by Kümmerer et al. (2016) has shown that the consumption of different antineoplastic drugs differs from one country to another (unit: $mg.d^{-1}.P^{-1}$), e.g. 0.013 in France, 0.012 in Germany, 0.003 in Austria, 0.56 in Switzerland and 0.002 in Denmark respectively. In Germany, the total consumption of these drugs was 22000 kg in the year 2001, 42000 kg in the year 2008 and 50000 kg in the year 2012. Cristóvão et al. (2020) has reported the consumption of several antineoplastic drugs in cancer hospitals of Portugal, Belgium and India and their predicted environmental concentration (PEC) values. According to this study, the consumption rate in Portugal was 177.2 kg in 2012 and 260.9 kg in 2016 of 101 antineoplastic drugs in the hospitals, respectively. However, in Belgium, the value was 2897.4 kg in 2012 and 3004.2 kg in 2015 for 99 antineoplastic drugs, while in India it was 6364 kg in 2016 for 33 antineoplastic drugs. These statistics clearly indicate that most of the antineoplastic drugs administered to cancer patients at hospitals are also excreted in hospital wastewater.

Verlicchi et al. (2020) reviewed the literature data for the presence of ~ 35 antineoplastic drugs in different water compartments, from the year 1990 to 2017, in eighteen different countries. According to this literature information, Aherne et al. (1990) monitored the levels of bleomycin in wastewater samples from the UK, while Steger-Hartmann et al. (1996), reported cyclophosphamide as well as IF levels in the hospital wastewater samples of Germany. On the other hand, ~ 28 antineoplastic drugs were detected in hospital wastewater, with their concentrations ranging from 2 to 266000 $ng.L^{-1}$. These included alkylating agents (0.85 to 266,000 $ng.L^{-1}$), antimetabolites (0.24 to 124000 $ng.L^{-1}$), plant alkaloids (2.75 to 99.70 $ng.L^{-1}$), hormonal agents (0.2 to 133.40 $ng.L^{-1}$) and antitumor antibiotics (5 to 21000 $ng.L^{-1}$), respectively. The following drugs were also reported: cyclophosphamide, IF, 5-FU, azathioprine, capecitabine, gemcitabine, methotrexate, tegafur, epirubicin, etoposide, irinotecan, docetaxel, paclitaxel, vincristine, tamoxifen, anastrozole, letrozole, erlotinib, etc., among others.

Table 2.2. Occurrence of antineoplastic compounds in samples of aquatic environment of different countries

Antineoplastic compounds	Country	Hospital effluent ($ng.L^{-1}$)	WWTP influent ($ng.L^{-1}$)	WWTP effluent ($ng.L^{-1}$)	Surface water ($ng.L^{-1}$)	References
Ifosfamide	Germany	6-1914	6-29	6-43	-	(Kümmerer et al., 1997)
		-	14.6	-	0.05-0.014	(Buerge et al., 2006)
		-	-	10-2900	<10	(Ternes, 1998)
		-	-	-	-	(Ternes et al., 2005)
	Spain	Nd-228	Nd-130	-	-	(Ferrando Climent, 2016)
		-	Nd-27.9	Nd-15.9	-	(Negreira et al., 2014a)
		-	3.5	1.2	-	(Martín et al., 2011)
	China	4-10647	-	-	-	(Yin et al., 2010)

	Slovenia	48-6800	-	-	-	(Česen et al., 2015)
Tamoxifen	China	0.2-8.2	0.28	-	-	(Liu et al., 2010)
	U.K.	-	-	-	27-212	(Roberts and Thomas, 2006)
	Spain	-	110-147	Nd-180.6	-	(Negreira et al., 2014a)
		26-170	Nd-58	11-42	25-38	(Ferrando Climent, 2016)
	France	-	-	<102	<25	(Coetsier et al., 2009)
Methotrexate	Spain	Nd-19	Nd-26	Nd-6	-	(Ferrando Climent, 2016)
	China	2-4689	-	-	-	(Yin et al., 2010)
Cyclophosphamide	Spain	5300	13100	-	-	(Gómez-Canela et al., 2012)

	-	Nd-43.8	Nd-25	-	(Negreira et al., 2014a)
	Blq-200.7	Nd-26	7-25	-	(Ferrando Climent, 2016)
Germany	146	-	-	-	(Steger-Hartmann et al., 1996)
	19-4500	6-143	6-17	-	(Steger-Hartmann et al., 1997)
	-	-	10-20	<10	(Ternes, 1998)
	-	-	-	-	(Ternes, 1998)
Switzerland	-	2-11	-	0.05-0.17	(Buerge et al., 2006)
Slovenia	14-22000	19-27	17	-	(Česen et al., 2015)
China	6-2000	-	-	-	(Yin et al., 2010)

	France	30-900	-	300	-	(Catastini et al., 2008)
Cytarabine	Spain	-	9.2	14	13	(Martín et al., 2011)
Etoposide	Spain	-	15	3.4	-	(Martín et al., 2011)
		Nd-714	Nd-175	-	-	(Ferrando Climent, 2016)
	China	5-380	-	-	-	(Yin et al., 2010)
Gemcitabine	Spain	-	9.3	7.0	2.4	(Martín et al., 2011)
	Switzerland	<0.9-38	-	-	-	(Kovalova et al., 2009)
5-Flourouracil	Austria	20000-122000	-	-	-	(Mahnik et al., 2007)
	Switzerland	<5-27	-	-	-	(Kovalova et al., 2009)
	Slovenia	35-92	4.7-14	-	-	(Kosjek et al., 2013)

Bleomycin	France	<30	-	-	-	(Catastini et al., 2008)
Oxaliplatin	Iran	170000	-	-	-	(Ghafuria et al., 2018)
Doxorubicin	Spain	-	4.5	-	-	(Martín et al., 2011)
	Austria	-	260-1350	-	-	(Mahnik et al., 2007)
Platinum compounds	Austria	3000-250000	-	-	-	(Lenz et al., 2007)
		1700	-	-	-	(Hann et al., 2005)
	France	350	-	-	-	(Goullé et al., 2012)
	UK	-	-	20-140000	-	(Vyas et al., 2014)
		-	-	30-100000	-	
Daunorubicin	Austria	<60	-	-	-	(Mahnik et al., 2007)
Docetaxel	Spain	Nd-98	Nd-219	-	-	(Ferrando Climent, 2016)

Epirubicin	Spain		-	24800	-	(Gómez-Canela et al., 2012)
2', 2'-difluorodeoxyuridine	Switzerland	<9-840	-	-	-	(Kovalova et al., 2009)
Doxorubicinol	China	<10	-	-	-	(Yin et al., 2010)
Azathioprine	Spain	Blq-188	Nd-20	-	-	(Ferrando Climent, 2016)
	China	15	-	-	-	(Yin et al., 2010)
Capecitabine	Spain	-	8.2-27	-	-	(Negreira et al., 2013)
		-	Nd-72.6	Nd-36	-	(Negreira et al., 2014a)
Anastrozole	China	0.3-3.7	0.12-0.32	0.3	-	(Liu et al., 2010)
Vincristine	Spain	Nd-49	Nd-23	-	-	(Ferrando-Climent et al., 2014)

	China	<20	-	-	-	(Yin et al., 2010)
Hydroxy-tamoxifen	Spain	-	-	Nd-5.8	-	(Negreira et al., 2014a)
Vinorelbine	Spain	-	-	9.1	-	(Martín et al., 2011)
4-hydroxy-N desmethyltamoxifen	Spain	-	-	91.6	-	(Negreira et al., 2014a)
Paclitaxel	Spain	Blq-100	Nd-18	-	-	(Ferrando Climent, 2016)
Letrozole	China	0.20-2.38	0.28-0.8	0.27-0.60	-	(Liu et al., 2010)
Carboplatin	Iran	280000	-	-	-	(Ghafuria et al., 2018)
Procarbazine	China	<5	-	-	-	(Yin et al., 2010)
Irinotecan	Spain	-	Nd-21.3	Nd-16.8	-	(Negreira et al., 2014a)

Hydroxy-paclitaxel	Spain	-	Nd-18.5	Nd-3.7	-	(Negreira et al., 2014a)
Cisplatin	Slovenia	35.2	2.33	1.28	-	(Vidmar et al., 2015)
	Iran	193500	-	-	-	(Ghafuria et al., 2018)

Note: Blq = below limit of quantification; Nd = not detected

Besides, some of these patients who were receiving treatment at home (outpatients) contributed to the release of these drugs in household effluents. In France, about 86.2 % of the antineoplastic drugs were released from household effluent, while the rest (~ 13.8 %) were from the hospitals (Besse et al., 2012). Based on the review done by Verlicchi et al. (2020), 25 antineoplastic drugs were also reported to be present in municipal wastewaters and 22 in WWTP effluents, in concentration range from 0.12 to 144000 $ng.L^{-1}$ (Steger-Hartmann et al., 1996; Kümmerer et al., 1997; Mahnik et al., 2004; Tauxe-Wuersch et al., 2005; Mahnik et al., 2006; Mahnik et al., 2007; Lenz et al., 2007; Catastini et al., 2008; Weissbrodt et al., 2009; Liu et al., 2010; Yin et al., 2010; Verlicchi et al., 2012; Ferrando-Climent et al., 2013; Kosjek et al., 2013; Ferrando-Climent et al., 2014; Gómez-Canela et al., 2014; Negreira et al., 2014a; Vyas et al., 2014; Azuma et al., 2016; Isidori et al., 2016a). As expected, the concentration of these drugs in hospital wastewater was higher when compared to municipal wastewater. On the other hand, the WWTPs effluent contained higher concentration of these drugs when compared to WWTPs influents.

2.3. Toxicity of antineoplastic compounds

Antineoplastic drugs have an adverse effect on the genetic makeup and cell cycle of aquatic flora and fauna because of chronic exposure (Johnson et al., 2008; Rowney et al., 2009; Booker et al., 2014). Many authors acknowledge the fact that these drugs are pseudo-persistent pollutants (Jones, 2005; Hernando et al., 2006). A recent study has suggested that a lower concentration of antineoplastic drugs in the pollutant mixture will have the same toxic effect as a single dose in higher concentration (Elersek et al., 2016). The bioaccumulation and biomagnification processes may lead to high levels of antineoplastic drugs in the aquatic environment. Several studies have reported the toxicity of various antineoplastic drugs on different aquatic organisms and cell lines. The results are usually reported in the form of lowest observed effect concentration (LOEC), effective concentration (EC_{50}), lethal dose (LD_{50}) and inhibitory concentration (IC_{50}) values (Zounková et al., 2007). The mixture of antineoplastic drugs causes more potent DNA damage to non-target cells, even at low concentrations, when compared with the parent drug itself (Novak et al 2017). EC_{50} value of bleomycin and vincristine was found to be <10 $mg.L^{-1}$ and in the range of 10 to 100 $mg.L^{-1}$, respectively (Jureczko and Przystaś, 2019). Platinum based drugs such as cisplatin and carboplatin are commonly present in hospital effluents and they are considered to be highly toxic to aquatic organisms (Ghafuria et al., 2018; Aldossary, 2019). Similarly, cyclophosphamide and 5-FU have reported to cause mutagenic effects in tadpoles (da Costa Araújo et al., 2019). 5-FU, imatinib and cisplatin are the most potent drugs to cause transgenerational effects on certain aquatic species (Mišík et al., 2019). The recalcitrant property of antineoplastic drugs leads their passage from sewage treatment plants to the surface water in its active form (Kümmerer, 2001). The native or parent compounds, as well as the by-product form of these drugs can induce adverse effects on both aquatic species and human life, e.g. direct physiological effects, genetic material damage and immune system damage (Zounkova et al., 2010; Filipič, 2014). The

organism present in aquatic environment would come in contact with the residue of antineoplastic pollutants throughout its life span and it will tend to accumulate the biomagnified pollutant within its body (Ghafuri et al., 2018; Jureczko and Przystaś, 2019; Jureczko and Kalka, 2020) (Table 2.3). Zounkova et al. (2010) studied the ecotoxicity effects of three antineoplastic drugs and their metabolites, namely 5-FU, gemcitabine and cytarabine on *Daphnia magna*, *Desmodesmus subspicatus* and *Pseudomonas putida* and genotoxicity effect on *Salmonella choleraesius*. The metabolite of 5-FU is α-fluoro-β-alanine (FBAL), while the metabolite of cytarabine is uracil-1-β-D-arabinofuranoside (AraU) and gemcitabine metabolite is 2, 2 – difluorodeoxyuridine (dfdU), respectively. According to this study, the native/parent forms of these antineoplastic drugs were able to cause higher toxicity when compared to their metabolites. The metabolites showed less or no toxicity and among these metabolites only FBAL showed significant toxic effect on the aquatic organisms. Cesen et al. (2016a) investigated the genotoxicity and ecotoxicity effects of cyclophosphamide, IF and their metabolites, as a single compound and in mixtures. The ecotoxicity effects of the three metabolites of cyclophosphamide, namely N–dechloroethyl-cyclophosphamide, keto-cyclophosphamide and carboxy-cyclophosphamide were tested on *Pseudokirchneriella subcapitata* and *Synecococcus leopoliensis*, while the genotoxicity effect was tested on *Salmonella typhimurium*. According to the authors, interestingly, among these three metabolites, only carboxy-cyclophosphamide showed toxicity and the EC_{50} value for compound was 17.1 $mg.L^{-1}$ (low value: 14.4 $mg.L^{-1}$; high value: 20.2 $mg.L^{-1}$).

In another study (Calza et al., 2014), the degradation of methotrexate, doxorubicin and the toxicity of their transformed products were tested on *Vibrio fischeri*. During degradation, eight by-products (M_1-M_8) of methotrexate and twelve by-products (D_1-D_{12}) of doxorubicin were formed. In the case of methotrexate and doxorubicin, the initial transformed products showed high toxicity, while the other end-products of degradation showed less toxicity on *V. fischeri*.

Table 2.3. Toxicological assessment of different antineoplastic drugs on various organisms

Antineoplastic drug	Tested organism	Critical effect	Concentration ($mg.L^{-1}$)	Ecotoxicity	References
5-Flourouracil	*Vibrio fischeri*	Luminescence	0.12	EC_{50}	(Backhaus et al., 2000)
	Pimephales promelas	Growth	20	LOEC 120 h	(DeYoung et al., 1996)
	Daphnia magna	Reproduction	0.05	LOEC 21 days	(Zounkova et al., 2010)
	Daphnia magna	Reproduction	0.0028	NOEC 21 days	(Straub, 2010)
	Aphanizomenon flos-aquae	Growth	0.002	NOEC 72 h	
	Zebrafish liver cell line	Cell viability	0.01	LOEC 72 h	(Novak et al., 2017)
	Daphnia magna	Reproduction inhibition	20.84	EC_{50} 48 h	(Parrella et al., 2014a)
	Ceriodaphnia dubia		501	EC_{50} 24 h	
	Thamnocephalus platyurus		0.28	EC_{50} 24 h	
Paclitaxel	*Daphnia magna*	Immobilization	>0.074	EC_{50} 48 h	(CDER, 1996)

Cytarabine	*Daphnia magna*	Reproduction	3.7	LOEC 21 days	(Zounkova et al., 2010)
Erlotinib	*Selenastrum capricornutum*	Growth	0.14	NOEC 72 h	FASS (2011)
	Daphnia magna	Reproduction	0.7	NOEC 48 h	
	Oncorhynchus mykiss	Survival	0.02	NOEC 14 days	
Capecitabine	*Daphnia magna*	Reproduction	>850	EC_{50} 48 h	(Straub, 2010)
	Pseudokirchneriella subcapitata	Growth	0.14	NOEC 72 h	
	Vibrio fischeri	Luminescence	2.16	EC_{50} 15 min.	(Barisci et al., 2018)
	Daphnia magna	Reproduction inhibition	224	EC_{50} 48 h	(Parrella et al., 2014a)
	Cceriodaphnia dubia		123000	EC_{50} 24 h	
	Thamnocephalus platyurus		197.7	EC_{50} 24 h	
	Fish		566 AT, 56.9 CT	LC_{50} 48, 96 h	(Huo et al., 2020)
	Daphnia magna		486 AT, 52.3 CT	LC_{50} 48, 96 h	

	Green algae		0.897 AT, 22.1 CT	EC_{50} 96 h	
Thiotepa	*Daphnia magna*	Immobilization	546	EC_{50} 48 h	(CDER, 1996)
Gemcitabine	*Daphnia magna*	Reproduction	>1.0	LOEC 21 days	(Zounkova et al., 2010)
	Pseudokirchneriella subcapitata	Growth	0.57	EC_{50} 72 h	FASS (2011)
	Daphnia magna	Immobilization	>0.99	EC_{50} 48 h	
	Pimephales promelas	Survival	>1000	LC_{50} 96 h	
	Oncorhynchu mykiss	Survival	>1000	LC_{50} 96 h	
Cladribine	*Daphnia magna*	Immobilization	233	EC_{50} 48 h	(CDER, 1996)
Methotrexate	*Vibrio fischeri*	Luminescence	3.0	EC_{50} 15 min.	(Barışçı et al., 2018)
	Vibrio fischeri	Luminescence	1220	EC_{50}	(Henschel et al., 1997)
	Scenedesmus subspicatus	Growth	260	EC_{50} 72 h	
	Tetrahymena pyriformis	Growth	45	EC_{50} 48 h	

	Daphnia magna	Immobilization	>1000	EC_{50} 48 h	
	Brachydanio rerio	Survival	85	EC_{50} 96 h	
	Bluegill sunfish cells	Cell density	3	EC_{50}	
	Brachydanio rerio	Pulse rate	142	EC_{50} 48 h	
	Xenopus laevis	Growth	0.015	EC_{50} 96 h	(Bantle et al., 1994)
Doxorubicin	*Daphnia magna*	Reproduction inhibition	2.14	EC_{50} 48 h	(Parrella et al., 2014a)
	Ceriodaphnia dubia		5.18	EC_{50} 24 h	
	Thamnocephalus platyurus		0.31	EC_{50} 24 h	
Cyclophosphamide	*Daphnia magna*	Immobilization	>1000	EC_{50} 48 h	(Zounková et al., 2007)
	Pimephales subcapitata	Growth inhibition	930	EC_{50} 72 h	
	Zebra fish liver cell line	Cell viability	37.5	LOEC 72 h	(Novak et al., 2017)
Tamoxifen	PLHC-1 cell line	Cell viability	1.72	EC_{50} 24 h	(Caminada et al., 2008)

	PLHC-1 cell line		5.12		
	RTG-2 cell line		5.38		
	RTG-2 cell line		7.09		
	Pimephales promelas	F_1 growth	0.00001	112 days	(Williams et al., 2007)
	Pimephales promelas	F_1 larvae growth significant Decrease	0.00008	28 days	
	Pimephales promelas	Increase in vitellogenin in F_1 males	0.00001	112 days	
	Acartiatonsa	Larval development	49	EC_{50} 5 days	
	Selenastrum capricornutum	Growth	0.001	72 h	(Mater et al., 2014)
Letrozole	*Oryzias latipes*	Fecundity	0.005	LOEC 21 days	(Sun et al., 2007)
	Oryzias latipes	Fertility	0.005		
	Oryzias latipes	Increase in genotypic F_1 males	0.005		

Flutamide	*Brachionus calicyflorus*	Fertilization of sexual females	0.001	LOEC 96 h	(Preston and Snell, 2001)
	Gasterosteus aculeatus	Spiggin inhibition	0.5	LOEC 21 days	(Sebire et al., 2008)
	Gasterosteus aculeatus	Male behaviour	0.1		
	Pimephales promelas	Testis alterations	0.062	LOEC 21 days	(Jensen et al., 2004)
	Pimephales promelas	Increase of estradiol plasma levels	0.651		
Nilutamide	Green algae	Growth	1	NOEC	(CDER, 1996)
Bicalutamide	*Pimephales promelas*	Overall	0.01	NOEC	FASS (2011)
Ifosfamide	Zebra fish liver cell line	Cell viability	37.5	LOEC 72 h	(Novak et al., 2017)
Cisplatin	Zebra fish liver cell line	Cell viability	0.1	LOEC 72 h	(Novak et al., 2017)
	Daphnia magna	Reproduction inhibition	0.94	EC_{50} 48 h	(Parrella et al., 2014a)
	Ceriodaphnia dubia		2.50	EC_{50} 24 h	

	Tetrahymena platyurus		8.44	EC_{50} 24 h	
	Chlorella vulagris	Growth	106.2	IC_{50} 96 h	(Dehghanpour et al., 2020)
Carboplatin	*Chlorella vulagris*	Growth	124.3	IC_{50} 96 h	(Dehghanpour et al., 2020)
Oxaliplatin	*Chlorella vulagris*	Growth	153.9	IC_{50} 96 h	(Dehghanpour et al., 2020)
Bleomycin	*Lamna minor*	Growth inhibition	0.2	EC_{50} 7 days	(Jureczko and Przystaś, 2019)
	Daphnia magna		0.77	EC_{50} 48 h	
	Pseudomonas putida		7.27	EC_{50} 16 h	
Vincristine	*Lamna minor*	Growth inhibition	>100	EC_{50} 7 days	(Jureczko and Przystaś, 2019)
	Daphnia magna		7.74	EC_{50} 48 h	
	Pseudomonas putida		>100	EC_{50} 16 h	

Note: PLHC = Poeciliopsis lucida hepatocytes, RTG-2 = Rainbow trout gonad, EC = Effective concentration, AT = Acute toxicity, CT = Chronic toxicity, FASS = Federation of Animal Science Societies.

Similarly, toxicity studies of the parent antineoplastic drug and their metabolites have also been reported in the literature for capecitabine, cyclophosphamide, methotrexate and 5-FU on

different microorganisms (Lutterbeck et al., 2015b; Lutterbeck et al., 2016; Barışçı et al., 2018; Chen et al., 2019; Huo et al., 2020). The dispersion of antineoplastic drugs via drinking water has also been reported by Aherne et al. (1990). As an example, if a pregnant woman is undergoing chemotherapy, the antineoplastic drugs would have negative impact on the foetus with craniofacial and digital abnormalities because the anticancer drugs can cross the placenta (Paskulin et al., 2005; Jureczko and Kalka, 2020). There are very authentic rules which stipulate that the health care staff under the pregnancy period should be boycotted from the preparation and management of the cytotoxic drugs (Allwood et al., 2002).

Every beneficiary of the earth's food chain is a consumer of water and many of them directly depend upon the natural water bodies (Bai et al., 2018) such as lakes, rivers, bore wells and ponds. These water bodies are often contaminated with untreated domestic sewage (Zounkova et al., 2010). Researchers have found traces of numerous pesticides and other pharmaceutical compounds in the food chains *i.e*. food crops, fishes and seafood. Similar to these pesticides and pharmaceutical compounds, the antineoplastic drugs may also find their way to our food chain through fish or any kind of direct water body dependent foods. Antineoplastic agents work their way through the food chain by accumulating in the body of living organisms and becoming more concentrated as they move from one organism to another through the process of "biomagnification". The pharmaceutical compounds contaminate the surface water and extricate with time in a different niche of the aquatic food webs. However, there are very few scientific documents that report the presence of antineoplastic drugs in the food web. According to several imitated/custom-designed food web experiments, the gathering of antineoplastic drugs occurs at a greater magnitude in the life, ensuing at the lower tropic levels (e.g. algae) when compared to the higher trophic levels (e.g. fish) (Du et al., 2014; Ruhí et al., 2016). However, Xie et al. (2017) reported conflicting outcomes *i.e*. their study shows no accretion and tropic biomagnification of the drugs in the aquatic food webs. Hence, in order to

understand the generic motif of pharmaceutical compounds transmission in aquatic environments, long-term studies should be carried out because the bioaccumulation of these drugs depends on the species (Heynen, 2016; Lagesson et al., 2016).

2.4. Treatment technologies for removal of antineoplastic drugs

Several treatment technologies have been adopted by the researchers to mitigate antineoplastic drug pollution from the aquatic environment. Table 2.4 summarize the studies investigated for the mitigation of antineoplastic compounds. It can review the work done of the scientific community on the efficiency of different treatment technologies used for removal of antineoplastic compounds. Mainly three treatments process are used for the treatment of these compounds *i.e*. physiochemical, chemical and biological treatment and which involve different techniques for the treatment of antineoplastic compounds.

2.4.1. Physio-chemical treatment

The physiochemical treatment process involves membrane filtration and AOPs for the removal of antineoplastic compounds from wastewater. Membrane filtration involves both membrane separation and adsorption for the removal of pharmaceuticals compounds. Reverse osmosis membrane is one of the most comply used membrane separation process (Wang et al., 2009). While, in adsorption based separation processes, the use of powder activated carbon (PAC) is more efficient for long term column operations when compared to activated carbon loaded columns (Kovalova et al., 2013). This treatment strategy has the advantage of separating the water from the contaminants, thereby providing both water treatment and fulfilling the water demand. The performance of PAC and granulated activated carbon (GAC) depends on the K_{ow} value of the antineoplastic compounds and dose of adsorbent. Verlicchi et al. (2015) reported the removal of cyclophosphamide and IF by PAC from hospital wastewater, while Lenz et al. (2007) tested the removal of 5-FU and capecitabine from oncological ward effluent of a

hospital by GAC and showed the superiority of using GAC when compared to the use of PAC. Towards the treatment of pharmaceutical pollutants from wastewater, RO and NF (nanofiltration) membranes have also proven to be efficient. Wang et al. (2009) investigated the removal of cyclophosphamide by RO and NF and observed a rejection of around 90 %, which indicated that both NF-MBR (membrane bioreactor) and RO-MBR were highly efficient to remove cyclophosphamide from contaminated water. Besides membrane filtration, AOPs have also been tested for the removal of pharmaceutical/antineoplastic drugs present in wastewater, e.g. electron beam radiation, UV photolysis, and photocatalytic oxidation.

The removal efficiency of a particular treatment process for antineoplastic compounds varies and each method has its own specific advantage and limitation for a particular drug. For example, the removal efficiency of capecitabine in a UV irradiation process was 100 %, but the toxicity in aquatic system after treatment increased significantly due to the production of more toxic metabolites (Guo et al., 2015). 5-FU treated by UV/H_2O_2 process achieved 99.6 % degradation (Kosjek et al., 2013), while the cyclophosphamide degradation was only 90 % (Ferre-Aracil et al., 2016). In another study, the degradation of cytarabine was compared in different UV irradiation based technologies and the following results were achieved: UV/$S_2O_8^{2-}$ = 96 %, UV/H_2O_2 = 81 %, UV/$(OH)_4B_2O_4^{2-}$ = 65 %, and UV/C_3H_9COOH = 48 % (Ocampo-Pérez et al., 2016). Meanwhile, the treatment of methotrexate and doxorubicin by UV was only 60 % and 10 %, respectively, although both the compounds were removed completely by UV/TiO_2 (Calza et al., 2014). Lai et al. (2015), investigated the treatment of cyclophosphamide and IF by UV/TiO_2 and reported that, although the removal was ~ 100 %, the by-products formed during the treatment process showed higher toxicity when compared to the parent compound. These results were similar to the results achieved by Guo et al. (2015) for capecitabine degradation.

In two different studies, 5-FU and cyclophosphamide degradation was compared in a UV/H_2O_2, UV/TiO_2 and $UV/H_2O_2/Fe^{2+}$ system and the authors reported no toxicity of the treated effluent even when the removal efficiencies of 5-FU and cyclophosphamide were not > 99 % (Lutterbeck et al., 2015a; Lutterbeck et al., 2015b). Cesen et al. (2016b) as curtained the removal of cyclophosphamide and IF in a $UV/O_3/H_2O_2$ process and removal efficiencies > 98 % was reported for both cyclophosphamide and IF. Li et al. (2016) reported the degradation of busulfan, chlorambucil, cyclophosphamide, dacarbazine, flutamide, IF, tamoxifen and methotrexate by ozonation and the authors observed 100 % removal of chlorambucil, dacarbazine, flutamide, tamoxifen and methotrexate was achieved, 70 % removal for cyclophosphamide and IF, and no removal of busulfan.

In a combined O_3/H_2O_2 process, Ferre-Aracil et al. (2016) reported 100 % degradation of pharmaceutical drugs mixture, namely gemcitabine hydrochloride, temozolomide, methotrexate, hydroxy-methotrexate, irinotecan, imatinib, mesylate, IF, cyclophosphamide, erlotinib hydrochloride, etoposide, doxorubicin hydrochloride, capecitabine, endoxifen, 4-hydroxytamoxifen and tamoxifen citrate. Ferrando-Climent et al. (2017) demonstrated 100 % removal of tamoxifen in an UV/O_3 process and observed that the by-products formed during the degradation was more toxic compared to tamoxifen. As a novel treatment technique, the electro-degradation of cyclophosphamide and IF by boron doped diamond electrode was tested by Fabianska et al. (2015). In that study, the authors tested the effect of current density (4.8 to 16.0 mA cm^{-2}), IF concentration (5 to 55 $mg.L^{-1}$), and pH (4.0 to 9.5) and observed that the current density has a significant effect on the removal efficiency of both cyclophosphamide and IF, while pH did not affect the performance of the electro-degradation process.

2.4.2. Chemical treatment

The removal of antineoplastic drugs by chemical treatment processes was previously used in hospitals and pharmaceutical industries; however, they have been replaced recently by AOPs. In chemical treatment, different oxidizing agents such as sodium hypochlorite, potassium and sodium permanganate, Fenton reagent and hydrogen peroxide are used to remove various antineoplastic drugs like doxorubicin, epirubicin, cyclophosphamide, IF melphalan and idarubicin (Hansel et al., 1996; Castegnaro et al., 1997). During the chemical transformation of antineoplastic drugs, several by-products or intermediate chemicals are formed and these by-products are mutagenic in nature (Lutterbeck et al., 2015b). The conventional chemical treatment process includes methods such as neutralization, precipitation, ion exchange, disinfection (ozone, chlorine and UV) and adsorption (Tripathi et al., 2020).

In recent years, AOPs are commonly used for the removal or degradation of antineoplastic drugs. Typical examples of AOPs are ozonation, photo-assisted degradation, electrochemical oxidation and Fenton based degradation (Pieczyńska et al., 2017). These methods involve the use of free radicals for the degradation or transformation of the antineoplastic drug (Janssens et al., 2017). Briefly, the mechanism/steps of hydroxyl based AOPs can be summarized as follows: (i) the first step if the production of highly reactive free radicals (e.g. $OH^{\bullet}$), (ii) the $OH^{\bullet}$ has electrophilic functions and it can react with the antineoplastic drugs present in water, (iii) during the process, there is transfer of hydrogen ions and interaction of radicals, (iv) the $OH^{\bullet}$ have a very short lifetime and they are produced *In-situ* using oxidising agents such as H_2O_2 and O_3, irradiation (e.g. UV light source or ultrasound), and catalysts (e.g. Fe^{2+}), and (v) this process mineralizes the antineoplastic agents and it is transformed into less or non-toxic products in the water phase. In AOPs, many combined techniques have been developed for treating pharmaceutical compounds present in water: UV (O_3/UV), H_2O_2 (O_3/H_2O_2), and both (O_3/UV/H_2O_2). AOPs have proven to be effective in treating hospital wastewater (Lutterbeck

et al., 2015b; García et al., 2020). Many researchers have tested electrochemical oxidation (Hirose et al., 2005; Lazarova and Spendlingwimmer, 2008), ozonation, H_2O_2 and UV induced photo-oxidation for treating numerous recalcitrant pollutants present in water and wastewater (Roberts and Thomas, 2006; Broséus et al., 2009; Zhang et al., 2013b; Česen et al., 2015). Evidently, the literature reports have proven the effectiveness of different treatment processes, both as standalone and combined/integrated systems, and depending on the nature of the drug, variations in removal efficiencies has been observed. The operational parameters of the treatment system/reactor also affect the treatment efficiency, e.g. treatment time, concentration of the drug, initial pH, catalyst/adsorbent dose, nature of the oxidant, applied voltage/current intensity, etc, among others. However, the operating conditions and the degree of treatment achieved decides the final quality of the treated water, its toxicity and treatment costs. For example, although ozonation has proven to be efficient for removing a wide variety of pharmaceutical drugs, persisting organic pollutants, pesticides, insecticides/herbicides and volatile organics present in water, in most of the cases, the toxicity of the treated water is somewhat high than the initial pollutant (Lin et al., 2015). For the treatment of antineoplastic drugs, photo-assisted treatment has also shown promising results. In few studies, UV/H_2O_2 process has achieved 100 % removal, reduced toxicity of the treated water and increased biodegradability of the transformed products (Lutterbeck et al., 2015b; Lutterbeck et al., 2016; Koltsakidou et al., 2017). On the other hand, in some studies, although the photo-assisted process has shown 100 % efficiency for removing antineoplastic drugs, the toxicity of the by-products were shown to be high (Ocampo-Pérez et al., 2010; Lin and Lin, 2014; Ferrando-Climent et al., 2017).

Table 2.4. Different strategies used to mitigate antineoplastic drugs from wastewater samples

Strategies	Antineoplastic compounds	Source	Operating parameters	Conclusion	Reference
Biological treatment by WRF (*Trametes versicolor*, *Ganoderma lucidum)*	10 antineoplastic compounds namely (cyclophosphamide, Ifosfamide, ciprofloxacin, methotrexate, paclitaxel, azathioprine, etoposide, docetaxel, tamoxifen and vincristine)	Hospital wastewater	Kirk medium, 25 ºC temperature, 4.5 pH and 130 rpm for 9 days	Tamoxifen removed totally but cyclophosphamide and ifosfamide remains as such	(Ferrando-Climent et al., 2015)
	Cyclophosphamide and ifosfamide	Synthetic solution	Kirk medium + other nutrient source, 25 ºC temperature, 4.5 pH and 135 rpm for 6 days	Removal percentage for both cyclophosphamide and ifosfamide was less than 40 %	(Castellet-Rovira et al., 2018)
	Bleomycin and vincristine	Synthetic solution	Modified Kirk medium + 26 ºC temperature, 14 days	Bleomycin was removed 36 % and vincristine was removed 94 % after only 4 days	(Jureczko et al., 2021)

Membrane Bioreactor	Cyclophosphamide	Semi-synthetic wastewater	MBR volume 20 L, WWPF were 13.3 $L.d^{-1}$, HRT was 36 h, SRT was 20 days, DO level was kept between n 0 and 4.5 mg O_2 L^{-1}, temperature varied between 25 to 32 ºC and pH varied between 7 to 8	60 % removal efficiency	(Seira et al., 2016)
Enzymatic degradation (secreted by WRF)	Various antineoplastic compounds such as tamoxifen, ifosfamide, cyclophosphamide, etoposide etc.	Mixed sources	Growth medium + other nutrient elements, temperature range from 25 ºC to 35 ºC, pH range from 4 to 6 and degradation time period from 6 days to 30 days	More potential than white rot fungi	(Pereira et al., 2020)

Mixed approach (biological and photochemical)	16 antineoplastic compounds (cytarabine, gemcitabine, capecitabine, ifosfamide, cyclophosphamide, melphalan, chlorambucil, doxorubicin, daunorubicin, etoposide, irinotecan, vincristine, vinblastine, megestrol, prednisone and mycophenolic)	Hospital WWTPs	For photochemical – 22 ºC temperature, 6.5 pH and 48 h degradation time For biodegradation- 22 ºC temperature, 7.5 pH and 48 h degradation time	UV-H_2O_2 (eliminate all) > UV-C (not effective) > aerobic biodegradation (not capable)	(Franquet-Griell et al., 2017)
Activated sludge batch biotransformation	Vincristine	Synthetic wastewater used	9 days degradation time period, pH from 5 to 8 and activated sludge concentration 0.24 to 1.9 $g{\cdot}L^{-1}$	90 % removal of parent compound	(Kosjek et al., 2018)
MBR-Pilot (HRT- 24h)	5-Fluorouracil and anthracyclines (doxorubicin, epirubicin, daunorubicin)	Oncologic wastewater	MBR tank size 1000 L, HRL–260 $L.d^{-1}$, UV radiation - 254 nm and	5- Fluorouracil was readily biodegradable and adsorption to sludge was marginal, it could be completely eliminated	(Lenz et al., 2007)

			monitoring time period was 18 months	from the liquid phase; over 90 % of anthracyclines were removed mainly due to adsorption of suspended solids.	
MBR-Pilot	Cisplatin, carboplatin	Oncologic wastewater	MBR tank size 1000 L, HRL (hydraulic load)-100-200 L d^{-1}, HRT- 20-24 h and monitoring time period was 98 days	Moderate elimination efficiency (51 % – 63 %) of total platinum was achieved; carboplatin showed relatively low adsorption to activated sludge and was mainly present as an intact drug in both influent and effluent.	(Mahnik et al., 2007)
	Cyclophosphamide and its human metabolites	Domestic wastewater with	MBR volume 20 L, 25 ºC to 32 ºC temperature, 7-8 pH, SRT- 50 and 70,	Cyclophosphamide removal was up to 80 %, however,	(Delgado et al., 2011)

		inoculated activated sludge	HRT- 48 and 32 and aeration cycle 2 minutes	residue cytotoxicity was measured in permeate.	
Nanofiltration, NF/Granular activated carbon	Cyclophosphamide	Pre-treated surface waters	Pre-treatment time period– 100 days, 7.5 pH and 6.7, DOC- 6 and 12, conductivity – 530 and 920, pump pressure- 25 bars and treatment time period- 4 days	NF rejection > 90 % when water recovery is only 10 % but at 20 % water recovery only 30 % rejection for CP.	(Verliefde et al., 2007)
NF/RO	Cyclophosphamide, ifosfamide, paclitaxel and etoposide	Ultrapure water and MBR effluent, synthetic	MBR volume - 400 ml, HRT- 48 h, SRT- 50 days, 7.5 to 8 pH, room temperature and trans-membrane pressure range	RO performed with more than 90 % rejection in compared to NF's poor rejection of 20 – 40 %.	(Wang et al., 2009; Cristóvão et al., 2019)

		urine and real secondary effluent	between $5{\cdot}10^{+5}$ to $25{\cdot}10^{+5}$ Pa 250 ml feed solution, minutes, 300 rpm, room temperature and treatment time period was 30 minutes	Rejection in NF for etoposide was maximum *i.e*. 97.7 and 98.7 % in ultrapure water and secondary effluent as compared to paclitaxel and IF.	
Electrolysis (anodic oxidation)	Epirubicin, irinotecan, vincristine, mitomycin-C, paclitaxel, methotrexate, cisplatin	Clinic wastewater	Two platinum-irridium electrodes (gap 5mm), 100 mA constant current and current density was 4 A dm^{-2}	Cytotoxicity, mutagenicity and antibacterial activity of epirubicin were ~ 100 % eliminated after electrolysis (6 h), 72 – 100 % for other investigated cytostatics.	(Hirose et al., 2005)

				The cost-effective apparatus can be adapted to treat clinical wastewater.	
Electrolysis (anodic oxidation)	Methotrexate	Urine	Platinum-irridium electrodes, 1 A constant current, 3.5 to 4 V voltage and electrolysis time period was 4 h	Electrolysis generates active chlorine and decomposes methotrexate	(Kobayashi et al., 2012)
Indirect photochemical degradation	Cyclophosphamide, ifosfamide	Lake water	pH 8.8, 2.51 mM alkalinity, 1.6 $mg.L^{-1}$ dissolved organic carbon, 22 ºC temperature foe light and 20 ºC for dark	Elimination efficiency was 80 % and 60 % for Cyclophosphamide and IF. Increased $OH^{\bullet}$ by adding nitrate enhanced the degradation of CP and IF.	
UV and UV/H_2O_2	Cyclophosphamide	Pure water and	8 W low-pressure mercury lamp–254 nm, H_2O_2	UV dose decreased from 5201 to 1695 mJ cm^{-2} (UV/H_2O_2) for	(Kim et al., 2009)

		biologically treated water	concentration was 6 to 8.2 $mg{\cdot}L^{-1}$, 20 ºC temperature and 7 pH	90 % Cyclophosphamide – degradation. H_2O_2 addition significantly enhanced CP degradation by UV radiation. The H_2O_2 enhancement was more effective to less readily-degraded PPCPs. Dissolved organic matters might act as scavengers of both $OH^{\bullet}$ and UV energy.	
Ozonation	Cyclophosphamide, methotrexate	Drinking water	Natural water (10 $mg{\cdot}L^{-1}$ O_3), natural water spiked with tert-butanol (10 $mg{\cdot}L^{-1}$ O_3), natural water (10 $mg{\cdot}L^{-1}$ O_3) spiked with hydrogen peroxide (2.5 10	CP degradation rate with O_3 was low ($ko_3 = 3.3{\pm}0.2\ M^{-1}s^{-1}$, pH 8.1) without significant natural water matrix effects; reaction of Cyclophosphamide with $OH^{\bullet}$ was much easier (k =	(Garcia-Ac et al., 2010)

			mg·L^{-1} H_2O_2), buffered ultrapure water (8.10 pH) spiked with tert-butanol (10 mg·L^{-1} O_3) and temperature was 20 ºC	2.0×10^9 $M^{-1}s^{-1}$). A high concentration (oxidant dose × contact time) value of ~45 mg min/L was required to remove 96 % CP from natural water. By comparison, methotrexate reacted quickly with O_3 (ko_3 > 3.6×10^3 $M^{-1}s^{-1}$) at typical dosages applied in drinking water treatment.	
UV/H_2O_2/O_3 and its sub-processes	Cyclophosphamide	Deionized water sample	Reactor volume – 1 L, 0.45 kW medium pressure, polychromatic UV lamp-200-300nm and temperature was 25 ºC	ko_3 = 2.5 $M^{-1}s^{-1}$ (CP+O_3 in excess); k_{OH}• = 1.3×10^9 $M^{-1}s^{-1}$ (Cyclophosphamide + OH•). H_2O_2/O_3 show highest degradation rate among different AOP conditions.	(Lester et al., 2011)

Biological treatment systems have also been tested for the removal of pharmaceutical drugs present in water. Although, the operating costs for biological processes are less compared to physio-chemical processes. In order to achieve 100 % removal of the pharmaceutical drugs present in water, some laboratory scale studies have shown that, a combination of one or two technologies, e.g. UV/O_3 + biodegradation or UV/H_2O_2 + biodegradation, will be more efficient to meet discharge/regulatory limits (Lutterbeck et al., 2020).

2.4.3. Biological treatment

The biological treatment process involves the use of microorganisms (mixed or pure cultures) for the removal of persistent pharmaceuticals from water. Currently, researchers have reported that the conventional biological wastewater treatment processes are not able to efficiently remove or degrade these compounds (Franquet-Griell et al., 2017; Castellet-Rovira et al., 2018; da Rosa et al., 2019). On the other hand, WRF emerged as promising tool for the removal of recalcitrant pharmaceuticals from the aquatic environment. WRF can secrete extracellular and intracellular oxidoreductase or ligninolytic enzymes. These enzymes can effectively degrade wide range of pharmaceuticals including antineoplastic compounds (Haroune et al., 2014; Ferrando-Climent et al., 2015; Castellet-Rovira et al., 2018; Singh et al., 2018; Pereira et al., 2020). Several modes have been used by the researcher for the removal of antineoplastic drugs by WRF or their oxidoreductase enzymes which are as follows:

1) Whole-cell culture
2) Enzymatic treatment

2.4.3.1. *Whole-cell culture*

Some studies investigated on the removal of antineoplastic compounds by the use of whole cell culture of WRF (Table 2.5). The whole-cell culture involved the use of fungi mycelium into solid or liquid medium under different culture conditions such as temperature, pH and shaking.

In general, the temperature range of whole cell treatment of WRF was between 25-35 ºC, pH range was 4.5-5.5 and rpm range for shaking was 90-200. In most of the degradation studies, glucose was used as carbon source (Asif et al., 2017). Ferrando-Climent et al. (2015) as curtained the biodegradation of tamoxifen, etoposide, cyclophosphamide and IF by the fungi *T. versicolor* and *G. lucidum* in a synthetic solution and non-sterile hospital wastewater, respectively. They reported that, the removal of tamoxifen was 48 % in non-sterile hospital wastewater and 99 % in the synthetic solution, respectively. In the case of IF and etoposide, the removals rate was 40 and 100 % in non-sterile hospital wastewater. In another similar study, Castellet-Rovira et al. (2018) tested the removal of cyclophosphamide and IF using *T. versicolor* and reported very less removal of the drugs (*i.e.* ~ 40 %). MBR with high biomass concentration and retention time have also proven to be effective for the biodegradation and removal of pharmaceutical drugs (Martín et al., 2011). Seira et al. (2016) tested efficiency of a MBR for the removal of a cytostatic drug (e.g. cyclophosphamide) and characterized the mechanism (adsorption/biodegradation) of pollutant removal. The authors operated a 20 L MBR for 153 days, at an inlet cyclophosphamide concentration of 5 $\mu g.L^{-1}$ and reported ~ 60 % cyclophosphamide removal. Jureczko et al. (2021) reported biodegradation of two antineoplastic drugs vincristine and bleomycin by six different WRF *i.e. F. fomentarius, H. fasciculare, P. nidulans, P. ostreatus and T. versicolor*. They compared the degradation rate of these fungi for vincristine and bleomycin. In case of vincristine, the degradation was only achieved by three fungi *F. fomentarius, H. fasciculare* and *T. versicolor* with 94-97 % degradation rate. However, in bleomycin, the degradation was shown by two fungi and the rate shown by *T. versicolor* was 36 % *and H. fasciculare* was 25 %.

2.4.3.2. *Enzymatic treatment*

WRF can produce extra and intracellular oxidoreductase ligninolytic enzymes which are laccase, manganese peroxidase, lignin peroxidase (extracellular), CYP_{450} and nitro-reductase

(intracellular). Every WRF species are not able to produce all these extracellular enzymes. Some fungi such as *T. versicolor, P. chrysosporium, G. lucidum* and *B. adusta* can secrete all three enzymes extracellularly (Asif et al., 2017). The rate of specific enzyme production also influenced by growth medium compositions and culture conditions. As the whole cell treatment need long reaction time and having chance of contamination, the use of crude enzyme provides short reaction time, reduce the chance of contamination and provide less toxic transformed products after degradation (Pereira et al., 2020). Several study investigated the use of cell free enzyme and immobilized enzyme to reduce the operation time of treatment process. Kelbert et al. (2021) reported the treatment of antineoplastic compound doxorubicin by the use of laccase enzyme. They reported the highest degradation of enzyme at pH 7.0 and 30 ºC temperature. In addition to antineoplastic compound degradation, several other recalcitrant pharmaceuticals were also degraded by crude enzyme secreted by different WRF. Lignin peroxidase was extracted by *P. chrysosporium* used for the removal of diclofenac and the removal rate was 100 % (Zhang and Geißen, 2010). Laccase was used for removal of estrone extracted by *T. versicolor* and the removal rate was 100 % (Auriol et al., 2007). Manganese peroxidase was used for the degradation of tetracycline and oxytetracycline extracted by *P. chrysosporium*. The removal rate of tetracycline was 72 % and oxytetracycline was 84 % (Wen et al., 2010). Versatile peroxidase was used for the degradation of diclofenac, estrone and naproxen extracted by *B. adusta*. The removal rate for diclofenac was 100 %, estrone was 100 % and naproxen was 80 % respectively (Eibes et al., 2011).

The intercellular mechanism for degradation of recalcitrant micropollutants is mediated by the CYP_{450} in coordination or without co-ordination with the extracellular system. The unseen involvement of CYP_{450} in the transformation of recalcitrant phenolic or aromatic compounds have been revealed in different studies. The intracellular system is also necessary for transforming different xenobiotics compounds in fungi (Olicón-Hernández et al., 2017).

Intracellular enzyme Cytochrome P_{450} monooxygenase can also play a key role in degradation of recalcitrant pharmaceutical compounds. It can degrade pharmaceuticals or phenolic compounds by oxygenation, halogenation reaction inside the fungal cell (Golan-Rozen et al., 2011).

2.5. Removal mechanism

Fungi adopt several pathways to counteract with a myriad of toxic or hazardous compounds such as recalcitrant polycyclic aromatic hydrocarbons, pesticides etc. They can follow mainly two pathways for the removal process *i.e.* non-enzymatic pathway (bio-adsorption, bio-precipitation) and enzymatic pathway (biotransformation and biodegradation that are mediated by enzymatic systems). Bio-adsorption is mediated by the specific composition of the cell wall such as chitosan or chitin (Asif et al., 2017; Grelska and Noszczyńska, 2020). In some fungi, such as *Phoma* sp. UHH 5-1-03, biosorption into fungal mycelia has an important role for bisphenol A, 17a-ethinylestradiol and triclosan removal, until this bio-adsorption reaches equilibrium (Pezzella et al., 2017). Biotransformation process is mediated by enzymes. Hydroxylation can be regarded as a biotransformation strategy for bioremediation processes, since this reaction can increase the solubility of pollutants and thereby reduce the bioaccumulation potential. Biotransformation of recalcitrant micropollutants include hydroxylation, oxidation sulfoxidation and dealkylation reactions (Eibes et al., 2011; Wang et al., 2013; Xiao and Kondo, 2020).

In addition to biosorption, there are other factors, including pollutant structure, fungal species, enzyme systems, culture medium, pH, temperature and enhancing methods e.g. the presence of mediators that affects the removal performance of a WRF (Mir-Tutusaus et al., 2018). *T. versicolor* fungus secrete three extracellular enzymes (lignin peroxidase, laccase, manganese peroxidase) and laccase is the predominant in some strains (Grelska and Noszczyńska, 2020). This fungus grows well in aqueous media than on solid matrices that can be due to better mass

transfer on liquid media. Furthermore, the degradation efficiency is not similar in all strain of fungi for a particular compound. Several other properties of WRF make them better in removal of different pharmaceutical compounds such as (Asif et al., 2017):

- Non-specificity of their produced enzyme that make them degrade a wide range of micropollutants
- Fast colonization through hyphal growth that allow fungus to access more pollutants
- Production and secretion of enzymes to degrade compounds with low water solubility
- Ability to treat pharmaceutical compounds in different range of pH.

The removal efficiency of WRF can be higher in non-sterile matrices than in sterile conditions due to the consortium established. Additionally, in non-sterile matrices bacteria could degrade the most biodegradable transformation products of the toxic compounds transformed by the WRF (Mir-Tutusaus et al., 2016). On the other hand, non-sterility reduces the duration of bioreactor operation due to native microorganisms exerting competitive pressure in WRF survival. This aspect has been partly resolved by introducing a pre-treatment step that reduces the initial concentration of microorganisms in the influent (Mir-Tutusaus et al., 2016; Mir-Tutusaus et al., 2018). From this perspective, several studies have focused on the use of whole cell basidiomycetes fungi, especially *T. versicolor*, to optimize degradation conditions as well as to implement new techniques for the monitoring of recalcitrant micropollutants. However, the filamentous growth could have operational problems associated (clogging, fouling and problems for biomass separation). The removal mechanisms involved in treatment with WRF whole-cell-culture can be divided into three steps including biosorption onto biomass, biodegradation through extracellular enzymes and intracellular or mycelium-bound enzymes (Asif et al., 2017; Naghdi et al., 2018; Pereira et al., 2020).

Table 2.5. Recent studies performed on the removal of antineoplastic compounds by whole-cell culture of white rot fungi.

Name of white-rot-fungi	Antineoplastic compounds	Operating conditions	Total removal efficiency (%)	Reference
Fomes fomentarius	Vincristine	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	97 (94 at 4 day)	(Jureczko et al., 2021)
	Bleomycin	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	No removal	
Hypholoma fasciculare	Vincristine	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	97 (94 at 4 day)	
	Bleomycin	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	58.5 (25 at 9 day)	
Trametes versicolor	Vincristine	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	97 (94 at 4 day)	
	Bleomycin	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	64.1 (36 at 9 day)	
	Tamoxifen	In real wastewater sample- Initial conc. 44.5 ng. L^{-1}, 4.5 pH, 25 ºC temp., 9 days In synthetic sample- Initial conc. 0.3 mg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 9 days	48 and 99	(Ferrando-Climent et al., 2015)
	Etoposide	In real wastewater sample- Initial conc. 197.5 ng. L^{-1}, 4.5 pH, 25 ºC temp., 9 days	100	

	Azathioprine	In real wastewater sample- Initial conc. 55 ng.L^{-1}, 4.5 pH, 25 ºC temp., 9 days	100	
	Cyclophosphamide	Initial conc. 10 mg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 9 days	No removal	
	Ifosfamide	In real wastewater sample- Initial conc. 77.2 ng. L^{-1}, 4.5 pH, 25 ºC temp., 9 days In synthetic sample- Initial conc. 10 mg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 9 days	61, No removal	
	Cyclophosphamide	Initial conc. 43.5 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	< 40	(Castellet-Rovira et al., 2018)
	Ifosfamide	Initial conc. 39.1 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	25	
	Iopromide	Initial conc. 174.4 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	47	
Pleurotus oestrus	Vincristine	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	52.5	(Jureczko et al., 2021)
	Bleomycin	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	No removal	

Phyllotopsis niulans	Vincristine	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	No removal	
	Bleomycin	Initial conc. 10 mg.L^{-1}, 26 ºC temp., 14 days	No removal	
Irpex lacteus	Cyclophosphamide	Initial conc. 43.5 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	23	(Castellet-Rovira et al., 2018)
	Ifosfamide	Initial conc. 39.1 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	23	
	Iopromide	Initial conc. 174.4 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	25	
Ganoderma lucidum	Cyclophosphamide	Initial conc. 43.5 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	> 40	(Castellet-Rovira et al., 2018)
	Ifosfamide	Initial conc. 39.1 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	< 40	
	Iopromide	Initial conc. 174.4 µg.L^{-1}, 25 ºC temp., 4.5 pH, 135 rpm, 6 days	> 30	

Materials and Methods

3.1. Materials

In this study all consumables (chemicals, reagents, glass wares, plastic wares, culture medium ingredients, biological kits and raw materials) were procured from Hi-Media (India), SRL (India), Merck (India), TCI (India), Sigma-Aldrich (India), Tarson (India) and Borosil (India). The chemicals used in this study are given in the Table 3.1.

Table 3.1. List of chemical components used in the experimentation and analysis of present study

Sr. No.	Chemical component	Make
1	Acetonitrile	Merck
2	HPLC water	Merck
3	Methanol	Merck
4	MTT	Merck
5	Etoposide	TCI
6	Paclitaxel	TCI
7	Cyclophosphamide	Hi-media
8	Urea	Hi-media
9	Ascorbic acid	Hi-media
10	Sodium bicarbonate	Hi-media
11	KCl	Hi-media
12	H_2O_2	Hi-media
13	Na_2HPO_4	Hi-media
14	Creatinine	Hi-media
15	KH_2PO_4	Hi-media
16	NaOH	Hi-media

17	Citric acid	Hi-media
18	DMEM	Hi-media
19	Foetal bovine serum	Hi-media
20	Penicillin-Streptomycin	Hi-media
21	Trypan blue	Hi-media
22	DMSO	Hi-media
23	Formic acid	Hi-media
24	Glucose	Hi-media
25	Ammonium tartrate	Hi-media
26	Yeast extract	Hi-media
27	Peptone	Hi-media
28	$CaCl_2$. $2H_2O$	Hi-media
29	$MgSO_4.7H_2O$	Hi-media
30	Thiamine-HCl	Hi-media
31	Nitriloacetate	Hi-media
32	$MnSO_4.2H_2O$	Hi-media
33	$ZnSO_4.7H_2O$	Hi-media
34	$CuSO_4.7H_2O$	Hi-media
35	$FeSO_4.7H_2O$	Hi-media
36	$Na_2MoO_4.2H_2O$	Hi-media
37	ABTS	Hi-media
38	Veratryl alcohol	Hi-media
39	Phenol red	Hi-media
40	Bovine serum albumin	Hi-media
41	Sodium lactate	Hi-media
42	Sodium tartrate	Hi-media

43	Potato dextrose broth	Hi-media
44	Sodium acetate	Hi-media
45	Potato dextrose agar	Hi-media
46	Agar-Type I	Hi-media
47	H_2SO_4	Hi-media
48	NaCl	Hi-media
49	Trypsin- EDTA	Hi-media
50	DNSA	Hi-media

The selected antineoplastic compounds are reported highly toxic in nature, so their handling during the experimentation require proper safety precaution such as use of nitril gloves, wear mask and lab coat. All the stocks of these antineoplastic compounds were prepared in fume hood containing disposal paper on the work surface to protect the surface from their exposure. After the preparation of stock, discard all the tubes and surface paper of hood that were in contact with these compounds in a separate biohazard container.

3.2. Microorganisms and Cell line

Ganoderma lucidum (MTCC-1039), *Phanerochaete chrysosporium* (MTCC-787) were collected from Microbial Type Culture Collection (MTCC), CSIR-IMTECH Chandigarh (India) in lyophilized form and *Trametes versicolor* (AH05) was obtained from Dr. Rajeev Kapoor Laboratory, Maharishi Dayanand University, Rohtak (Haryana) on agar petri plate form. These fungi are white rot fungi considered as microorganisms of Biosafety Level 1 (BSL-1). These are not reported for causing disease in individuals during handling them, and not able cause any hazardous effect to environment and laboratory staff. It is recommended that standard microbiological precautions must be employed while doing experiments with these microorganisms (Buchan et al., 2019).

The cell line Raw 264.7 was obtained from National Centre for Cell Science (NCCS), Pune (India) and Prof. Gobardhan Das, Special Centre for Molecular Medicine (SCMM), Jawaharlal Nehru University, New Delhi, India. This cell line is mouse monocyte macrophage or immune cell line. This is not reported for causing any disease in individual handling but to prevent contamination during their growth and media exchange it require proper handling and precautions.

3.3. Development of high-performance liquid chromatography methods

Here, three different revere phase HPLC methods for the detection and quantification of cyclophosphamide, etoposide and paclitaxel were developed (Fig. 3.1). Initially, three different stock solution of cyclophosphamide and etoposide were prepared according to their solubility. Cyclophosphamide and etoposide were prepared in acetonitrile, while paclitaxel was prepared in methanol and finally diluted in HPLC grade water. The final concentration of stock solution was 1000 $\mu g.ml^{-1}$ for each compound. After the preparation of stock of antineoplastic compounds, synthetic urine was prepared. The chemical compositions of synthetic urine are given in Table 3.2. Further, the antineoplastic compounds from the prepared stocks were spiked with synthetic urine and serially diluted in various concentrations *viz.* 10, 50, 100, 250, 500, 750 $\mu g.ml^{-1}$ for the determination of the LOD and LOQ. All the prepared solutions were filtered through 0.22 µm syringe filter prior to use in HPLC. The prepared stocks solutions were kept in dark condition at -20 ºC temperature for further analysis or use.

3.3.1. Optimization of prepared method

The chromatographic analysis was carried out on HPLC (CECIL Instrument Service Ltd., U.K) system with C_{18} column (250mm×4.6mm×5µm) using acetonitrile and water as mobile phase in different ratio at both gradient and isocratic mode for cyclophosphamide, etoposide and paclitaxel. Prior going to HPLC run, we scanned the prepared solution for each compound

using spectrophotometer to check the absorbance wavelength (nm) at which the compounds were giving maximum absorbance. For gradient as well as isocratic mode, different parameters were optimized. On the basis of best efficiency, the mode of run *i.e.* isocratic mode was selected for further selection and optimization of conditions. Finally, in isocratic mode the total run time, flow rate and temperature were optimized (Table 3.3).

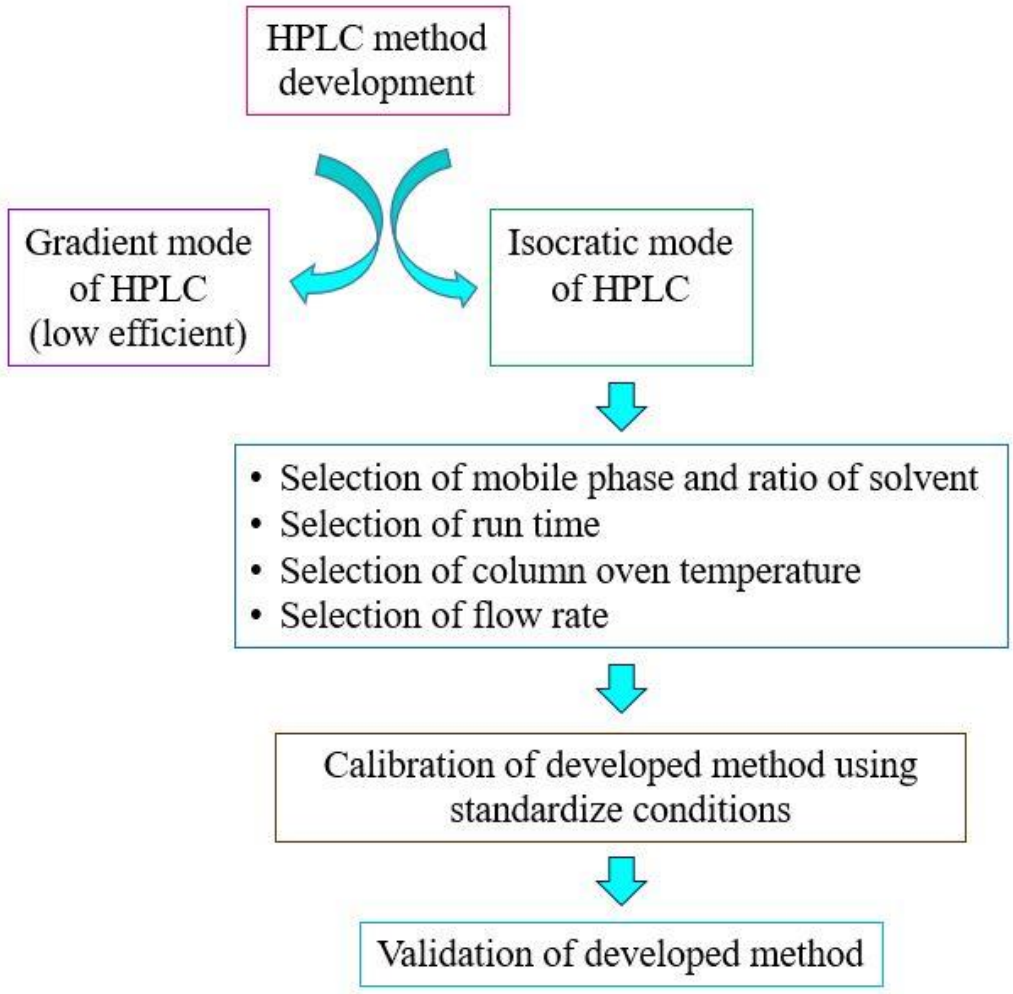


Fig. 3.1. Workflow of HPLC method development for selected antineoplastic compounds

Table 3.2. Chemical composition of synthetic urine (Calafat and Sampson, 2012)

Sr. no.	Chemical component	Quantity used ($g.L^{-1}$)
1	KCl	3.8
2	NaCl	8.5
3	Urea	24.5
4	Ascorbic acid	0.34
5	KH_2PO_4	1.18
6	NaOH	0.64
7	Citric acid	1.03
8	Sodium bicarbonate	0.47
9	Creatinine	1.4
10	H_2SO_4	28 ml

Table 3.3. Selection of conditions under isocratic mode for detection and quantification of cyclophosphamide, etoposide and paclitaxel in HPLC

Time (min.)	Mobile phase (Acetonitrile:Water)	Flow Rate ($ml.min.^{-1}$)	Temperature (^0C)	Wavelength (nm)
	Cyclophosphamide			
10	50:50, 60:40, 70:30, 80:20, 90:10	0.8, 1.0, 1.2	20, 25, 30	199
	Etoposide			
10	50:50, 60:40, 70:30, 80:20, 90:10	0.8, 1.0, 1.2	20, 25, 30	229
	Paclitaxel			
10	50:50, 60:40, 70:30, 80:20, 90:10	0.8, 1.0, 1.2	20, 25, 30	229 nm

3.3.2. Validation of developed methods

The developed methods were validated by the measurement of different characteristics such as accuracy, sensitivity range, specificity, linearity and robustness.

3.3.2.1. *Linearity, Accuracy and Precision*

To check the linearity of the method developed, we had serially diluted the cyclophosphamide, etoposide and paclitaxel stock solution in synthetic urine to achieve different concentration *i.e.* 10, 50, 100, 250, 500 and 750 $\mu g.ml^{-1}$. Each diluent with respective standard concentration injected into triplicate under optimized condition. The linearity was evaluated by calculation of linear regression (r^2) from the plot between peak area and concentration. The accuracy of the developed method was calculated by the recovery of sample at their different concentration level for cyclophosphamide, etoposide and paclitaxel. The precision of the developed methods was assessed by repeatability testing using standard solution of 500 $\mu g.ml^{-1}$ of cyclophosphamide, etoposide and paclitaxel analysed ten times.

3.3.2.2. *Sensitivity, Specificity and Robustness*

The sensitivity was determined by measuring the signal to noise ratio of LOD and LOQ of test sample in HPLC. The robustness of the method developed was evaluated by changing different parameters such as column temperature, flow rate and variation ratio of mobile phase. The optimized column temperature range was 20, 25 and 30 ºC, flow rate was 0.8, 1.0 and 1.2 $ml.min^{-1}$ and mobile phase ratio of acetonitrile and water was 90:10, 80:20, 70:30, 60:40 and 50:50. Under these optimized parameters, the peak area, peak height and retention were calculated. The specificity was evaluated by the comparing the chromatogram of standard sample and blank.

3.4. Growth of selected fungal culture

Two fungi *G. lucidum* and *P. chrysosporium* culture were present in the form of lyophilized powder in glass vial. Glass vials were broken in biosafety cabinet (Labtech Laminar Air Flow Systems, New Delhi, India). The *G. lucidum* cultured was inoculated on MTCC growth medium (65) containing malt extract 10 $g.L^{-1}$ and agar 20 $g.L^{-1}$ in double distilled water. The pH of culture medium was adjusted at 6.5 ±0.1 before sterilization. *P. chrysosporium* was inoculated on MTCC growth medium (43) containing malt extract 20 $g.L^{-1}$, glucose 10 $g.L^{-1}$, peptone 01 $g.L^{-1}$ and agar 20 $g.L^{-1}$ in double distilled water. *T. versicolor* was sub-cultured on malt extract agar 20 $g.L^{-1}$. Sterilization was conducted in autoclave machine. After inoculation, these fungi were incubated at 25 ^{o}C temperature in incubator under static mode for 5 days.

3.4.1. Mycelium pellet formation

After the growth of fungi on petri plates, a part or slice of fungal colonies from the growing petri plates were transferred into broth medium containing 2 % malt extract, 10 % glucose and 4.5 pH. All fungi were grown at 25 ^{o}C and 150 rpm for 7 days. After 7 days of incubation, mycelium pellets of fungi were formed. Then, the pellet of each fungus was filtered through autoclaved muslin cloth containing jar under aseptic environment and washed with autoclaved deionized water. After this, the pellet was stored in 0.8 % saline (0.2 µm syringe filtered) at 4 ^{o}C for further use.

3.5. Optimization of culture condition for biomass production

In biomass production, we have optimized three parameters *i.e.* pH, temperature and agitation to produce high amount of biomass. The pH range selected for the optimization of optimum condition was from 3.5 to 5.5 in the interval of 0.5, the temperature range was from 24 ^{o}C to 32 ^{o}C in the interval of 2 ^{o}C, while the agitation range for shaking condition was from 90 to 210 in the interval of 30 rpm.

Initially, about 1.5 $g.L^{-1}$ of dry weight of mycelium pellet was inoculated into defined growth medium (100 ml) and grown in shaking incubator under selected condition. The chemical compositions of defined growth medium are given in Table 3.4 (Kirk et al., 1978; Wen et al., 2010; Castellet-Rovira et al., 2018). After 3, 6, 9, 12 and 15^{th} day of inoculation, the biomass of each fungus was determined by dry weight method. Here, the biomass from the flask of growing culture was filtered through Whatman filter and kept into glass plate in hot air oven at 60 ºC for overnight (Fig. 3.2). After drying of biomass, weight of the biomass measured on weighing balance to analyse the initial biomass size.

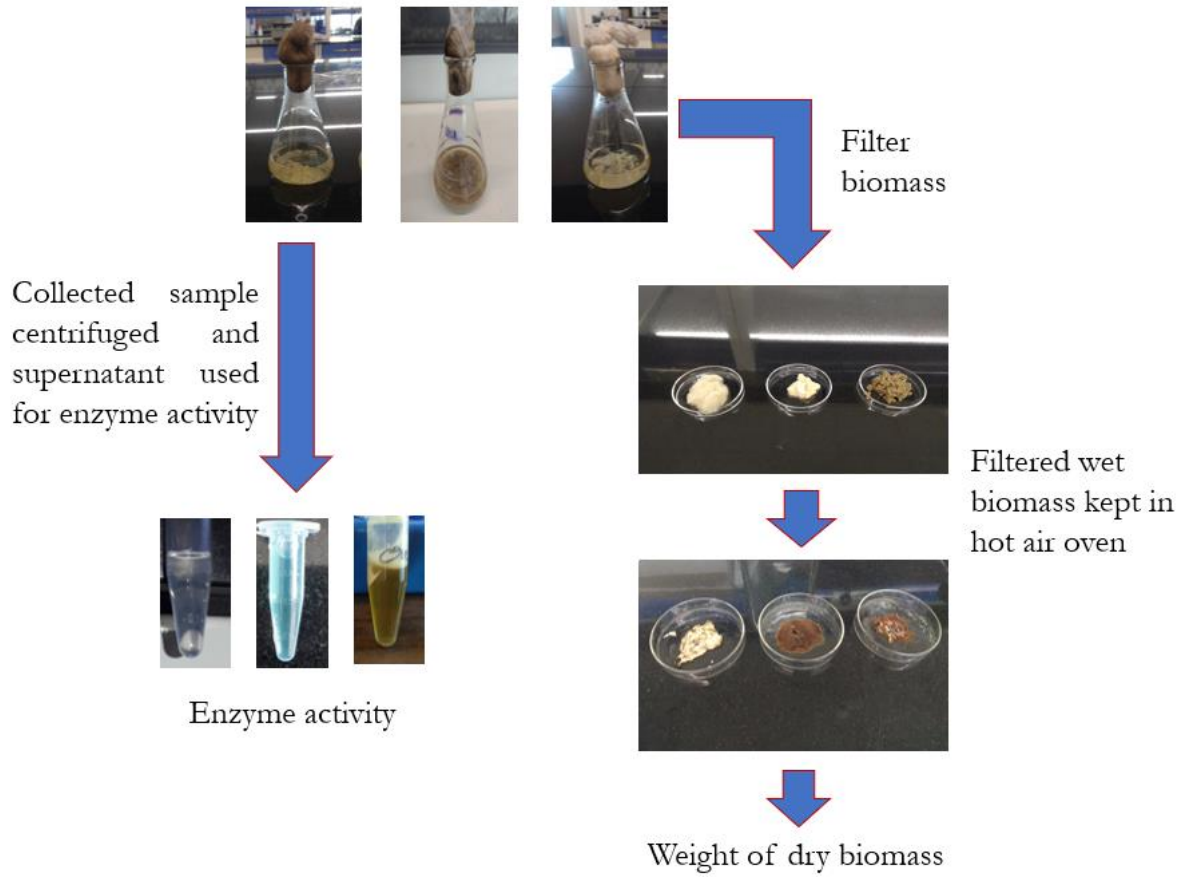


Fig. 3.2. Overview of analysis of dry weight biomass and extracellular enzyme activity estimation of selected fungi

Table 3.4. Chemical composition of growth medium for *G. lucidum, T. versicolor* and *P. chrysosporium*

Sr. no.	Chemical component	Concentration used ($g.L^{-1}$)
1	Glucose	10
2	Ammonium tartrate	2.0
3	Yeast extract	2.0
5	Peptone	2.0
6	KH_2PO_4	2.0
7	$MgSO_4.7H_2O$	0.5
8	$CaCl_2.2H_2O$	0.1
9	Thiamine-HCl	1 mg
	Trace element	
10	Nitriloacetate	0.3
11	$MnSO_4.2H_2O$	0.05
12	$ZnSO_4.7H_2O$	0.043
13	$CuSO_4.7H_2O$	0.05
14	$FeSO_4.7H_2O$	0.01
15	$Na_2MoO_4.2H_2O$	0.01
16	NaCl	0.1

3.6. Analysis of extracellular ligninolytic enzymes

On the basis of best optimized condition of biomass production, the activities of these enzymes were measured on 3, 6, 9, 12 and 15th days. At zero/initial day, the enzyme activity was not detected. Here, the sample from the test and control flasks were periodically taken out on 3, 6, 9, 12 and 15th days. 2 ml supernatant was taken from each cultured flask aseptically and filtered

through Whatman filter paper and after the filtration, sample was centrifuged at 10000 rpm for 10 minutes. Now, the supernatant obtained after centrifugation was used as enzyme for activity in different selected assay. The reaction mixture of enzyme assay are given in Table 3.5.

Table 3.5. Chemical composition and reaction mixture used for enzyme activity assay

Enzyme activity assay	Reagent	Stock concentration	Volume used in enzyme assay (in ml)
Laccase	ABTS	0.5 mM	0.1
	Sodium acetate buffer	0.1 M	0.3 ml
Manganese peroxidase	Phenol red	0.01 %	0.1
	Sodium lactate	0.25 mM	0.1
	$MnSO_4$	2 mM	0.05
	BSA	0.5 %	0.2
	H_2O_2	20 mM	0.05
	NaOH	2 N	0.04
Lignin peroxidase	Veratryl alcohol	10 mM	0.25
	Sodium tartrate buffer	125 mM	0.5
	H_2O_2	2 mM	0.25

Laccase assay

Add 0.6 ml enzyme extract, 0.1 ml ABTS and 0.3 ml sodium acetate buffer, pH 5.0 in 2 ml centrifuge tube and incubated at 30 ºC. The reaction mixture was incubated according to the adopted method and the absorbance was taken at UV-Vis spectrophotometer at 420 nm. In this assay, ABTS act as substrate for laccase (Papinutti et al., 2003).

Manganese peroxidase assay

Add 0.5 ml enzyme extract, 0.05 ml phenol red, 0.1 ml sodium lactate, 0.05 ml $MnSO_4$, 0.2 ml BSA, 0.05 ml H_2O_2 and 0.04 ml NaOH, 4.5 pH in 2 ml centrifuge tube and incubated at 30 ºC. The reaction mixture was incubated according to the adopted method and the absorbance was taken at UV-Vis spectrophotometer at 610 nm. In this assay, phenol red act as substrate for manganese peroxidase (Kuhawara et al., 1984).

Lignin peroxidase assay

0.25 ml enzyme extract, 0.25 ml veratryl alcohol, 0.5 ml sodium tartrate buffer and 0.25 ml H_2O_2 in 2 ml centrifuge tube and incubated at 30 ºC. The reaction mixture was incubated according to the adopted method and the absorbance was taken at UV-Vis spectrophotometer at 310 nm. In this assay, veratryl alcohol act as substrate for lignin peroxidase (Arora and Gill, 2001).

The enzyme activity for these enzymes was expressed as $IU.L^{-1}$. One unit is defined as 1 µmol substrate oxidized per min. by the enzyme (Wen et al., 2010).

3.7. Biodegradation of selected antineoplastic compounds by white rot fungi

Initially, an individual stock solution of three antineoplastic compounds (cyclophosphamide, etoposide and paclitaxel) were prepared of 300 $mg.L^{-1}$ concentration separately and filtered through 0.22 µm syringe filter. Then, each drug from stock solution was spiked with the defined medium under aseptic condition. The final concentration of drugs in each treatment and control flask was 300 $\mu g.L^{-1}$. In last, 1.5 $g.L^{-1}$ (dry weight) of fungal mycelium was inoculated in the experimental flasks.

The degradation experiment flasks were divided into 3 control *i.e.* one for blank or negative control (medium + antineoplastic compound spiked) to asses physiochemical degradation of

spiked compound, second for heat killed or abiotic control (medium + heat killed fungus + antineoplastic compound spiked) to asses the biosorption process rate of fungi for antineoplastic compound and third for biotic or experimental control (medium + antineoplastic compound spiked + live WRF inoculated) to asses degradation of drugs by fungi (Castellet-Rovira et al., 2018). In heat killed control fungi were inoculated before the autoclave of medium.

The experiment was run for continuously 15 days for each fungal culture. All flasks (experiment and control) were run in triplicate. The experiment flasks were run on different culture condition for each fungus according to their best optimized culture condition for production of biomass end extracellular enzyme. Here, *T. versicolor* treatment flasks were run at 25 ºC, 4.5 pH and 150 rpm, *G. lucidum* treatment flasks were run at 30 ºC, 5.0 pH and 150 rpm and *P. chrysosporium* treatment flask were run at 30 ºC, 4.5 pH and 180 rpm for 15 days in shaking incubator under dark to prevent photodegradation. However, on 0, 3, 6, 9, 12 and 15 days of inoculation, 1 ml sample from each experiment and control flasks were taken out to analyse the degradation rate of antineoplastic compounds by fungi on LC-MS.

Simultaneously, with biodegradation experiment the effect of cyclophosphamide, etoposide and paclitaxel on the growth of fungi were also analysed. Here, another control was used *i.e.* biomass control (defined medium + fungi), which incubated in the same conditions as mentioned above for degradation of antineoplastic compound. In the last day of experiment, biomass of control and experimental flask were filtered and dried at 60 ºC temperature in hot air oven. After this, dry weight of biomass was calculated by the given formula to analyse the negative effect of antineoplastic drugs on fungal growth (Eq^{n}. 1).

$$Effect\ of\ antineoplastic\ compound\ on\ growth\ of\ fungi: Total\ dry\ weight\ biomass\ of\ control - Total\ dry\ weight\ biomass\ of\ experimental\ flask \quad (1)$$

3.8. LC-MS analysis

For the degradation analysis, at 0, 3, 6, 9, 12 and 15 days of incubation 1 ml sample were taken from each experiment and control flask. Each sample was filtered through 0.22 µm syringe filter before injecting into LC-MS for further analysis. After the filtration of sample, the quantification of cyclophosphamide and etoposide were carried out in LC-MS instrument at Advanced Instrument Research Facility at Jawaharlal Nehru University New Delhi, India. During the detection of antineoplastic compounds concentration in LC-MS, three data set were assessed for the analysis of removal during treatment process which are given below in the following equation 2, 3 and 4:

$$Total\ removal\ rate\ (\%): \frac{Concentration\ of\ drugs\ at\ 0\ days - Concentration\ of\ drugs\ at\ 15th\ day\ in\ biotic\ control}{Concentration\ of\ drugs\ at\ 0\ day} \times 100 \quad (2)$$

$$Total\ sorption\ rate\ (\%): \frac{Concentration\ of\ drugs\ at\ 0\ days - Concentration\ of\ drugs\ at\ 15th\ day\ in\ heat\ killed\ control}{Concentration\ of\ drugs\ at\ 0\ day} \times 100 \quad (3)$$

$$Total\ biodegradation\ rate\ (\%): Total\ removal\ rate\ (\%) - Total\ sorption\ rate\ (\%) \quad (4)$$

3.9. Degradation kinetics study

The kinetics of degradation of cyclophosphamide, etoposide and paclitaxel by *G. lucidum*, *T. versicolor* and *P. chrysosporium* was investigated to analyse the half-time and removal rate. The degradation of these compounds followed the pseudo-first-order kinetics model to evaluate '$t_{1/2}$' and degradation rate. The following equation (5) was applied for this study (Bankole et al., 2020):

$$\ln\left(\frac{S0}{S}\right) = kt \quad (5)$$

where,

S_0 = substrate (antineoplastic compound) initial concentration

S = substrate concentration at time 't'

k = substrate degradation constant

3.9.1. Estimation of reducing sugar and pH

The glucose concentration and pH of the experimental flask was measured to predict the effect of these parameter on rate of degradation after variation in their set values. *G. lucidum, T. versicolor* and *P. chrysosporium* were grown in defined growth medium (Table 3.4), where the initial concentration of glucose in each flask was 10 $g.L^{-1}$. The consumption of glucose was analysed by the analysis of reducing sugar from the sample and it was estimated by DNS method (Fig. 3.3). DNS method involved DNSA (30 g of sodium potassium tartrate in 50 ml double distilled water, then 20 ml of 2 M NaOH added to it and mix gently. After this, 1 g of DNS added and final volume 100 ml was adjusted). During glucose estimation initially, sample from growing fungal culture was filtered through muslin cloth contained jar. Then, 2 ml of filtered sample taken in a test tube and 2 ml DNS reagent added to it. After this, test tube was kept in water bath at 100 ºC temperature for 10 min. (Fig. 3.3). In last sample was cool down at room temperature and the absorbance was taken at 540 nm (Garriga et al., 2017). The estimation of glucose was performed on 3, 6, 9, 12 and 15 days of experiment for each culture.

The pH value during biodegradation of antineoplastic compounds was measured at 0, 3, 6, 9, 12 and 15 days from the incubation start and compared the effect of pH variation in rate of degradation to their initial set value (Fig. 3.3).

The time-course-degradation profile of cyclophosphamide, etoposide and paclitaxel with each fungal culture were analysed on the basis of degradation rate with particular fungus and their pH and glucose levels presence in the experiment and control samples.

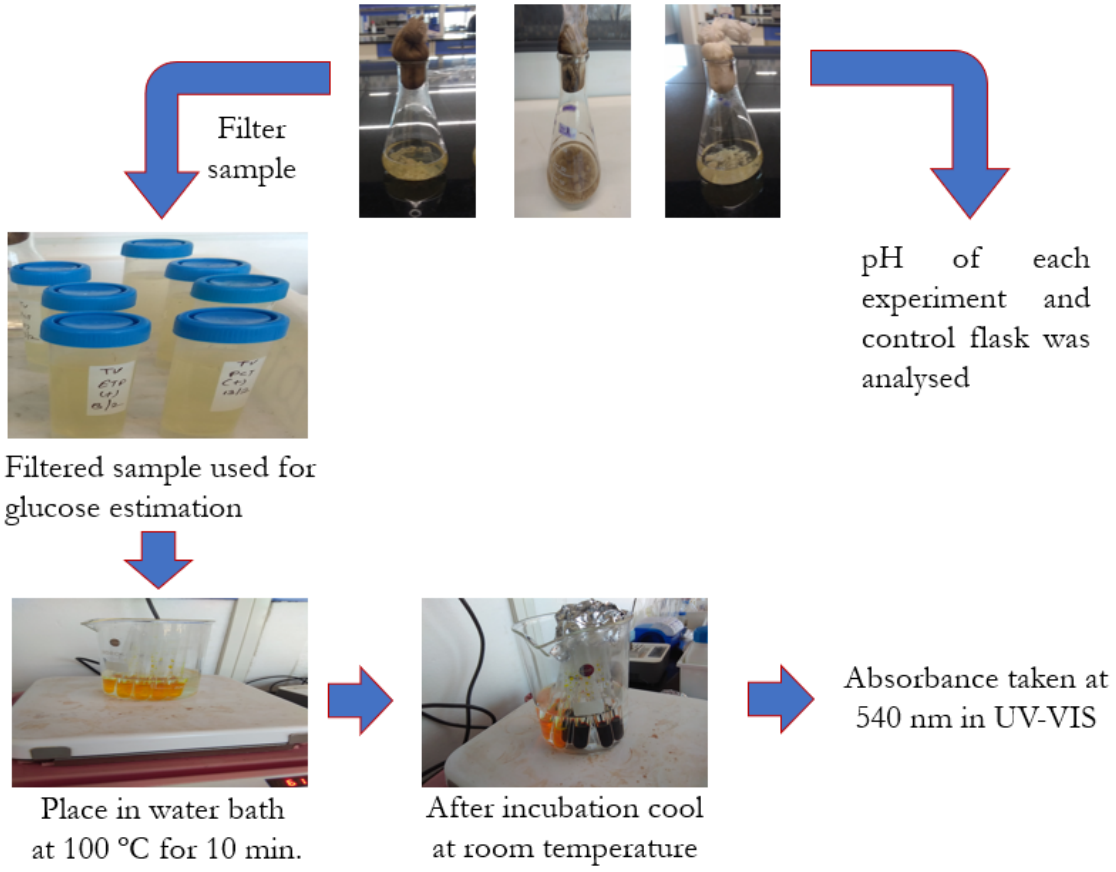


Fig. 3.3. Estimation of reducing sugar and pH of the *G. lucidum*, *T. versicolor* and *P. chrysosporium* during biodegradation experiment

3.10. Toxicity analysis

3.10.1. Cytotoxicity of native compounds

The cytotoxicity of selected antineoplastic was carried out on Raw 264.7 cell line. MTT assay was performed to analyse the cytotoxic effect of native forms of antineoplastic compounds. The 100 ml medium was prepared using the composition DMEM (10 $g.L^{-1}$), sodium bicarbonate (3.7 $g.L^{-1}$) and phenol red (15 $mg.L^{-1}$) dissolve into double distilled water. After the mixing of medium components, pH was adjusted to 7.2 to 7.4. Finally add, 11 ml FBS to make 10 % FBS containing medium and filtered the medium through filter assembly in sterile environment or biosafety cabinet-2 using vacuum pump. After the filtration, 1 ml from 100x stock penicillin-streptomycin solution into prepared medium was added, after this 1 ml medium

from freshly prepared medium flask was taken out and added into a 5 ml LB (Luria broth) tube. The medium was kept at 4 ℃ and LB tube at 37 ℃ temperature with 150 rpm in the incubator for overnight to contamination check. Beside the medium preparation, three different stock solution of cyclophosphamide, etoposide and paclitaxel were prepared of 1000 $\mu g.ml^{-1}$ in absolute DMSO and syringe filtered with 0.2 µm filter. After the preparation of stock of compounds, the dilution of stock was made into 250, 200, 150, 100, 50, 50, 25, and 10 $\mu g.ml^{-1}$ for each antineoplastic compound.

3.10.2. Cell seeding

After the sterility check of prepared medium, Raw 264.7 cells were grown in the prepared medium using a T-75 flask at 37 ℃ and 5 % CO_2 in an incubator (Das et al., 2012). When the sufficient growth of adherent cells achieved, the cells from the growing flask were washed out with the help of autoclaved 1x PBS in a sterile centrifuge tube and centrifuged at 2500 rpm for 5 min. for pellet collection. Then, remove the supernatant and mix the pellet gently by adding 5 ml of fresh media into tube. After the mixing of cells, take out 100 µl cell suspension and 400 µl of 0.4 % trypan blue. The cell suspension with trypan blue was used for cell count in the haemocytometer. The concentration or number of cells was counted by using diluent factor.

3.10.3. *In-vitro* cytotoxicity assay for native antineoplastic compounds

Primarily, 200 µl (1×10^4) of Raw 264.7 cells were seeded into each well of 96 well plate (flat bottom). The plate was kept in incubator at 37 ℃ with 5 % CO_2 for 24 hours. After 24 hours, plate was taken out from the incubator and 50 µl of drugs or compound sample (final concentration into well were 250, 200, 150, 100, 50, 25, and 10 $\mu g.ml^{-1}$) added into wells of the plate. DMSO was used as solvent control, the higher concentration (5000 $\mu g.ml^{-1}$) of antineoplastic compound was used as positive control and fresh medium was used as a negative control. Each well in the plate was containing the final volume 250 µl. All the controls and test

samples were added in triplicate into wells. After adding the sample, the plate was kept at 37 ℃ with 5 % CO_2 for 48 hours drugs treatment. After the 48 hours, medium from each well was removed and 200 µl fresh medium without FBS and 25 µl MTT (5 $mg.ml^{-1}$) were added. Then, the plate kept at 37 ℃ with 5 % CO_2 for 4 hours. After the 4 hours of incubation, MTT containing media from each well was removed and 200 µl DMSO into each well added as indicating in Fig 3.4. In last, the plate was covered with foil to avoid direct effects of light and shake in shaker for 15 minutes at 37 ℃ temperature. Finally, absorbance of the plate was measured at 590 nm in the microtitre plate reader.

The following equation was used to determine cytotoxicity of antineoplastic compounds:

$$\% \text{ of Cytotoxicity} = 100 - \left[\frac{\text{Absorbance (Sample)}}{\text{Absorbance (Control)}}\right] \times 100$$

The IC_{50} (Inhibitory concentration) of drugs were calculated by the equation $y = mx+c$ using MS-Excel software.

3.11. Toxicity of transformed products

Toxicity of intermediates or transformed products of cyclophosphamide, etoposide and paclitaxel formed during biodegradation were analysed by MTT assay same as used for cytotoxicity study of native form of these compounds with some modification. The toxicity was estimated to visualize the cytotoxic effect of transformed products on cell viability. During the biodegradation analysis experiment, simultaneously the samples for toxicity analysis of transformed products were also taken from the control and experiment flasks. 1 ml sample from each flask was taken and filtered through 0.22 µm syringe filter in biosafety cabinet under aseptic conditions. The filtered sample was used to determine the cytotoxicity of transformed products on cell viability of Raw 264.7 cells. The sample collected from normal growing fungus flask was used as negative control to assess the cytotoxic effect of metabolites produced by fungus during their life cycle and effect of medium composition presented in the flask.

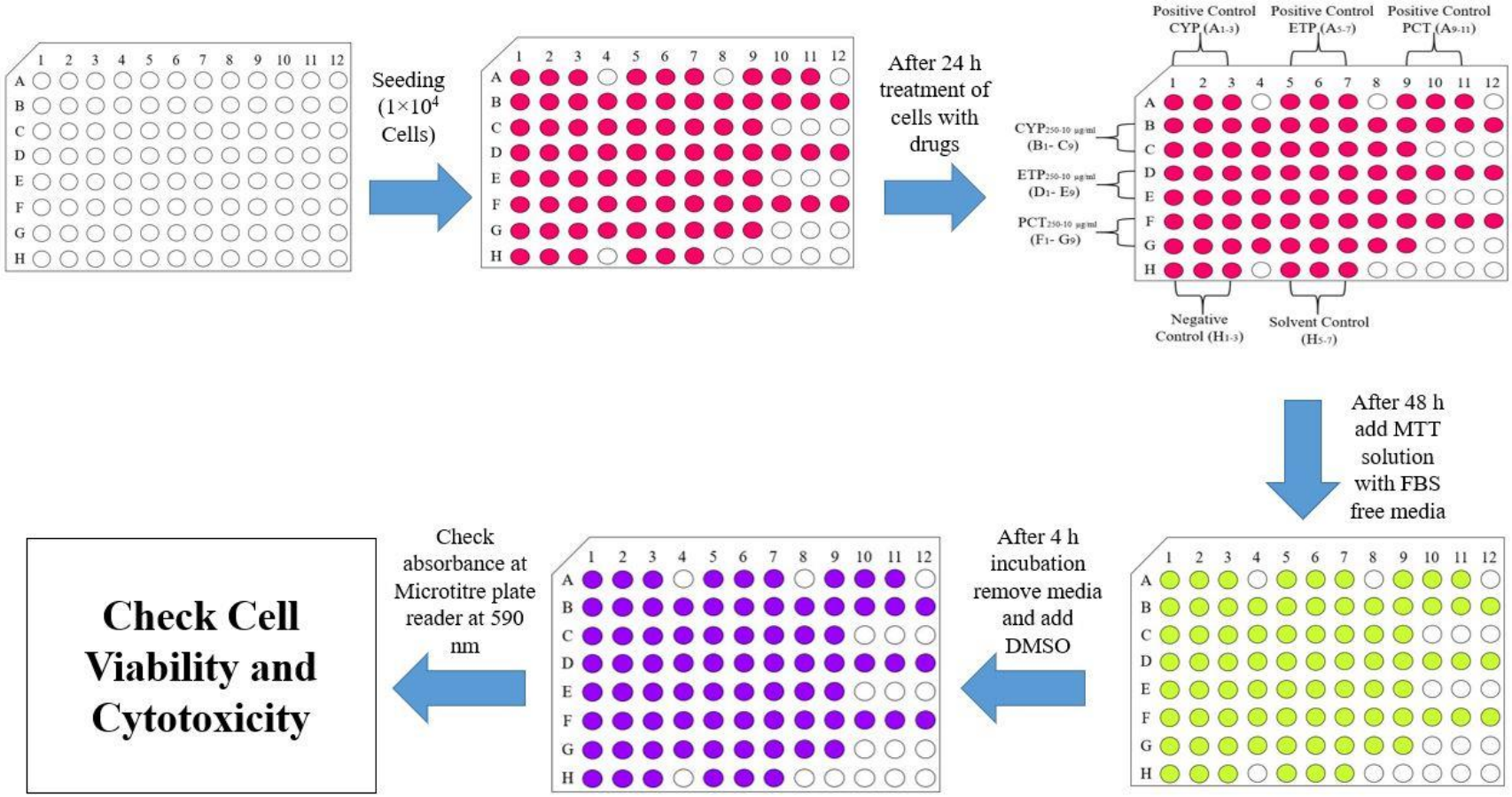


Fig 3.4. Schematic overview of *In-vitro* cytotoxicity analysis of native form of cyclophosphamide, etoposide and paclitaxel on Raw 264.7 cell line

Results and Discussion

4.1. Method development and standardization

In the development of HPLC methods for the detection and quantification of cyclophosphamide, etoposide and paclitaxel, we standardized the condition for the better elution of these compounds. Initially, we determined the maximum absorbance (ƛ max) of the target antineoplastic drug(s) by UV-Vis spectrophotometer. The ƛ max for cyclophosphamide after the scanning was 199 nm, while 229 nm for both paclitaxel and etoposide as given in Table 4.1. Further, we developed three different HPLC method using C_{18} column (250 mm×4.6 mm×5 µm) to detect and quantify the target antineoplastic compounds. The optimized mobile phase were acetonitrile and water (70:30) with 25 ºC column temperature and flow rate 1.0 $ml.min^{-1}$ at wavelength 199 nm for cyclophosphamide. The RT for best elution in case of cyclophosphamide was 03:32.3 ±0.41 min. (Table 4.2). While the optimized mobile phase for etoposide and paclitaxel detection were also acetonitrile and water in ratio 80:20 with 25 ºC column temperature at 229 nm wavelength and 1.0 $ml.min^{-1}$ flow rate. The retention time (RT) for best elution in case of etoposide and paclitaxel were 2:40.1 ±0.48 min. and 3:49.3±0.28 min. respectively. The run length for all these methods was 10 min. The optimized conditions and parameters were tabulated and presented in Table 4.2.

4.2. Validation of methods

4.2.1. Linearity, Accuracy and Precision

The linearity of the method is directly proportional to the correlation of the concentration of the drug molecule standard versus the result (concentration) obtained using the method developed for the same compound. The calibration graph shows the linear relationship between different concentration range of cyclophosphamide (10, 50, 100, 250, 500, 750 $\mu g.ml^{-1}$) with correlation coefficient (r^2) value 0.989872 (Fig. 4.1). Similarly, etoposide also shows the linear relationship between different concentration range (10, 50, 100, 250, 500, 750 $\mu g.ml^{-1}$) with r^2

value 0.999914 (Fig. 4.2). Fig. 4.3. shows the linear relationship between different concentration range of paclitaxel (10, 50, 100, 250, 500, 750 $\mu g.ml^{-1}$) with r^2 value 0.999788. All the test analytes cyclophosphamide, etoposide and paclitaxel show liner relationship and thus they have agreeable linearity 0.98, 0.99 and 0.99 respectively.

To calculate the accuracy, the percentage of reproducibility at all six concentration levels of cyclophosphamide, etoposide and paclitaxel peak area were calculated depicted in Table 4.3. This indicates the applicability and reliability of the method developed for analysis of these three antineoplastic compounds. The triplicate recovery data of each compound was similar at all the concertation analysed in term of peak area.

The precision of proposed method was measured by the repetition of homogenous sample concentration of paclitaxel, cyclophosphamide and etoposide under proposed condition and the results were tabulated and presented in Table 4.4. In this study, the peak area and retention time were analysed of a single concentration of each compound ten times repeats. The concentration used for precision validation was 500 $\mu g.ml^{-1}$. Here, among ten cases, as per the optimized RT 3:32 min. for cyclophosphamide observed similar in seven samples and peak area 1936 mAs observed in six sample, it was range from 1934 to 1939 mAs. These values shown not a large difference in peak area and RT. In etoposide, the optimized RT was 2:40 min. observed about similar in six samples and peak area was in range from 19408 to 194011 mAs. It was almost similar in all ten samples, there was no big difference in the peak area and RT of etoposide also. The optimized RT of paclitaxel was 3:49 min. was observed similar in six samples and peak area was observed in range from 18629 to 18630 in all ten samples. There was very close peak area and RT value in all cases.

Table 4.1. λ_{max} of cyclophosphamide, etoposide and paclitaxel absorbance by scanning on UV-VIS spectrophotometer

Sr. no.	Antineoplastic compound	Wavelength (In nm)	Maximum absorbance (OD)
1	Cyclophosphamide	199	2.223 ±0.008
2	Etoposide	229	3.650 ±0.035
3	Paclitaxel	229	3.954 ±0.013

Table 4.2. Optimized HPLC conditions for the detection of cyclophosphamide, etoposide and paclitaxel in water sample

Antineoplastic compound	Run Time (In min.)	Mobile Phase		Column	Column oven Temperature (° C)	Wavelength (In nm)	Retention time (RT) (in min.)	Co-relation cofficient (r^2 value)	Flow rate (ml.min.$^{-1}$)
		Acetonitrile (A)	Water (B)						
Cyclophosphamide	10	70	30	C_{18} (250mm×4.6mm×5µm)	25	199	3:32.3	0.989872	1.0
Etoposide	10	80	20	C_{18} (250mm×4.6mm×5µm)	25	229	2:40.1	0.999914	1.0
Paclitaxel	10	80	20	C_{18} (250mm×4.6mm×5µm)	25	229	3:49.3	0.999788	1.0

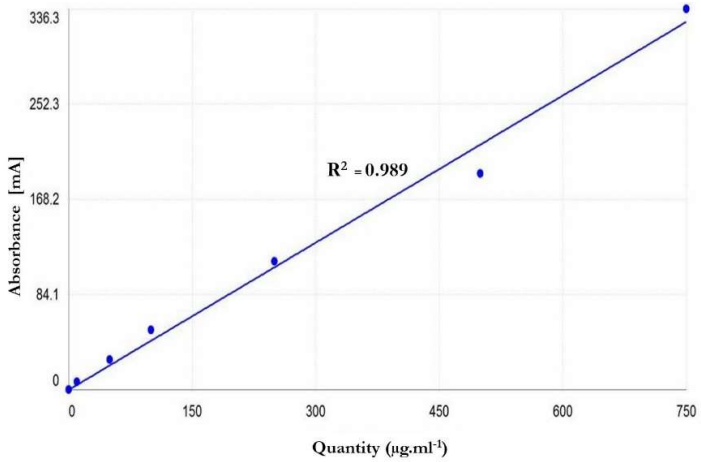


Fig. 4.1. Calibration graph of cyclophosphamide in HPLC at developed conditions

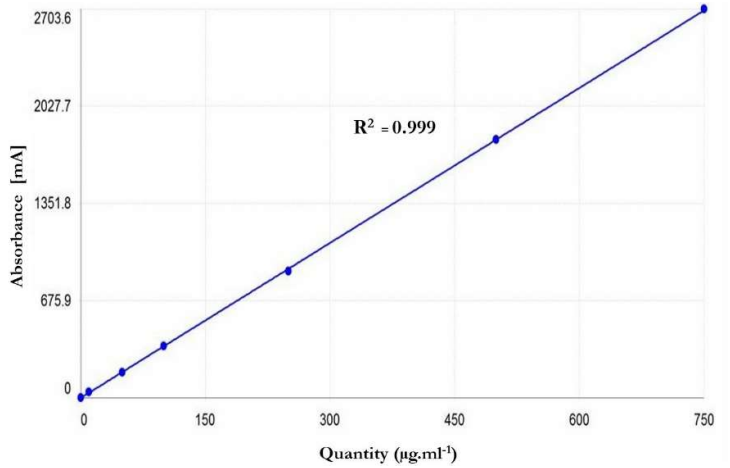


Fig. 4.2. Calibration graph of etoposide in HPLC at developed conditions

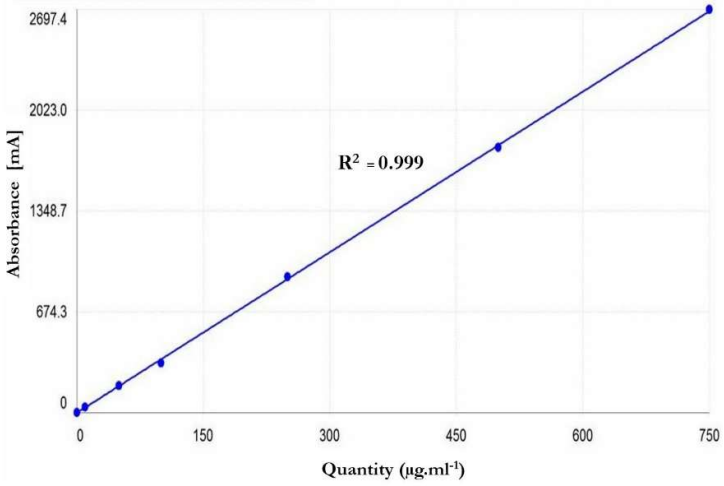


Fig. 4.3. Calibration graph of paclitaxel in HPLC at developed conditions

Table 4.3. Reproducibility data of cyclophosphamide, etoposide and paclitaxel in developed HPLC method

Name of compound	Spiked Level of compound (in µg.ml^{-1})	Replicate 1 (Peak area in mAs)	Replicate 2 (Peak area mAs)	Replicate 3 (Peak area mAs)
Cyclophosphamide	10	52.3	52.9	52.5
	50	272.9	274.4	273.1
	100	546.5	546.4	546.8
	250	1174	1174.2	1175.8
	500	1936.4	1937.2	1936.6
	750	2981.1	2996.5	2918.2
Etoposide	10	331.1	329.8	330.6
	50	1852.9	1850.7	1856.1
	100	3778.6	3754.5	3779
	250	9067	9072.2	9071.8
	500	18629.2	18632.4	18629.9
	750	26738.3	26738	26738.7
Paclitaxel	10	377.4	371.6	376
	50	1848	1848.6	1814.6
	100	3771.7	3752.1	3768.9
	250	10488.5	10479	10488.3
	500	19409	19409.7	19405.2
	750	31629.3	31632	31629.8

Table 4.4. Precision data of cyclophosphamide, etoposide and paclitaxel on developed HPLC methods

No. of Replicate	Cyclophosphamide (500 µg.ml^{-1})		Etoposide (500 µg.ml^{-1})		Paclitaxel (500 µg.ml^{-1})	
	Peak area (in mAs)	RT (in min.)	Peak area (in mAs)	RT (in min.)	Peak area (in mAs)	RT (in min.)
1	1936.1	3:32.2	18629.2	3:49.2	19409.2	2:40.5
2	1936.4	3:32	18630.4	3:49.3	19408.8	2:40.1
3	1934.6	3:32.8	18630.2	3:47	19408.9	2:41.8
4	1936.2	3:32.3	18629.3	3:49.6	19410.4	2:40.6
5	1937	3:36.1	18629.5	3:48.9	19407.7	2:40.4
6	1938.4	3:34	18629.6	3:49.2	19408.1	2:47.2
7	1935.4	3:32.4	18630.1	3:50.8	19409.7	2:41.2
8	1935.8	3:32.1	18629.8	3:49.1	19411.2	2:42
9	1934.8	3:33.4	18629.5	3:49.3	19409.6	2:40.5
10	1935.9	3:32.9	18629.9	3:50.6	19409.2	2:40.8

4.2.2. Sensitivity, Specificity and Robustness

The assessment of sensitivity of optimized method for cyclophosphamide, etoposide and paclitaxel was based on limit of detection *i.e.* 10 µg.ml^{-1} for each. These methods showing the minimum 10 µg.ml^{-1} of amount or concentration of above three antineoplastic compounds was detected and the limit of quantification was 1 µg.ml^{-1} or less.

The specificity of all three compounds was determined by comparing the blank and standard sample solutions by injecting 20 µl sample of blank and standard separately into the injecting loop of HPLC system. In Fig. 4.4, first chromatogram representing the blank or control chromatogram, while rest three chromatogram showing peak for cyclophosphamide of

concentration 250, 500 and 750 µg.ml^{-1} at RT 3:32.3 minutes. Similarly in Fig. 4.5, first chromatogram representing the blank or control chromatogram, while rest three chromatogram showing peak for etoposide of concentration 250, 500 and 750 µg.ml^{-1} at RT 2:40.1 minutes. In Fig. 4.6, first chromatogram representing the blank or control chromatogram, while rest three chromatogram showing peak for paclitaxel of concentration 250, 500 and 750 µg.ml^{-1} at RT 3:49.3 minutes. All these chromatograms indicated the purity of analytes peak and confirms the specificity of methods.

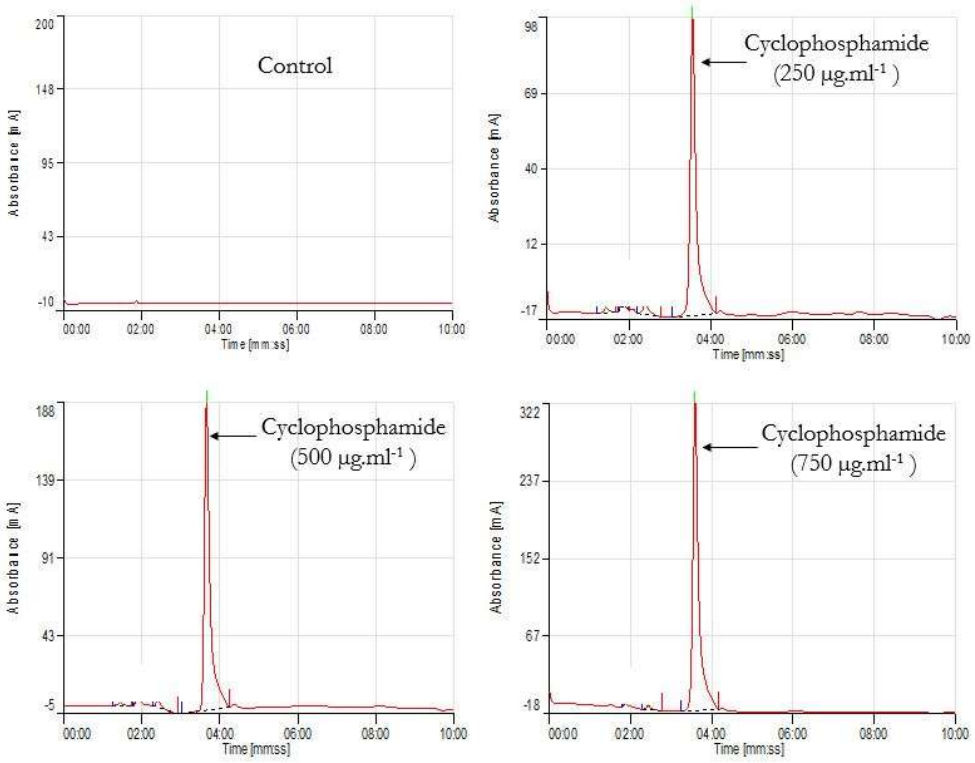


Fig. 4.4. HPLC chromatogram peak of cyclophosphamide for control and different concentration

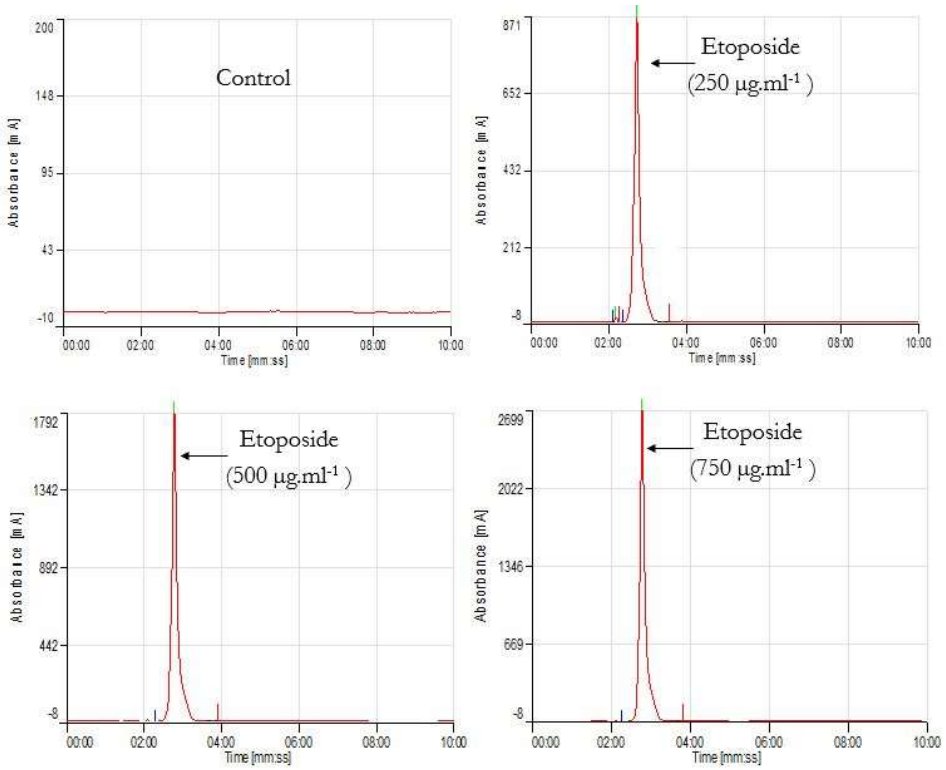


Fig. 4.5. HPLC chromatogram peak of etoposide for control and different concentration

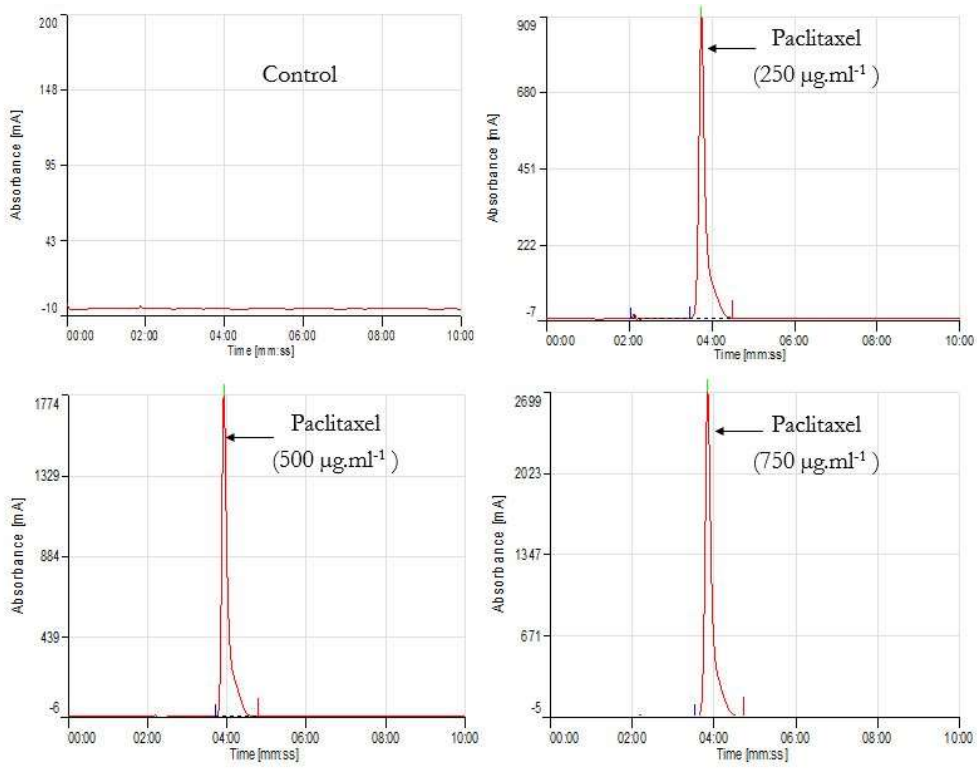


Fig. 4.6. HPLC chromatogram peak of paclitaxel for control and different concentration

The robustness of developed HPLC method was evaluated by changing the parameters and conditions of proposed method such as mobile phase, temperature, and flow rate and are summarized in Table 4.5. Here, the selected range of temperature was from 20 to 30 ºC, flow rate was 0.8 to 1.2 ml.min^{-1} and mobile phase ratio of acetonitrile and water was from 50:50 to 90:10. On these the parameters the peak area, peak height and RT of antineoplastic compound was optimized for each compound. The highest elution of cyclophosphamide was observed at 25 ºC temperature, 1.0 ml flow rate and 70:30 mobile phase, while in case of etoposide and paclitaxel was observed at 25 ºC temperature, 1.0 ml flow rate and 80:20 mobile phase. In other conditions the results were obtained for these antineoplastic compounds but their recovery or elution rate was very less as compared to the optimized conditions.

Table 4.5. Robustness of developed HPLC method for cyclophosphamide, etoposide and paclitaxel

Antineoplastic compound	Parameter optimized		Peak area (in mAs)	RT (in min.)
Cyclophosphamide	Temperature (ºC)	25	3431.1 ±0.26	3:32.3 ±0.41
	Flow rate (ml.min.$^{-1}$)	1.0		
	Mobile phase (Acetonitrile:Water)	70:30		
Etoposide	Temperature (ºC)	25	26738.3 ±0.33	2:40.1 ±0.48
	Flow rate (ml.min.$^{-1}$)	1.0		
	Mobile phase (Acetonitrile:Water)	80:20		
Paclitaxel	Temperature (ºC)	25	31629.9 ±0.36	3:49.3 ±0.28
	Flow rate (ml.min.$^{-1}$)	1.0		
	Mobile phase (Acetonitrile:Water)	80:20		

In 2003, Larson and his group developed HPLC method for the simultaneous analysis of cyclophosphamide and paclitaxel along with three other antineoplastic compounds (IF, 5-FU and doxorubicin) with the use of C_{18} column. The mobile phase used for this analysis were acetonitrile and water in combination with buffer in ratio of 22.75:77.25 at wavelength 195 nm and 1.2 $ml.min.^{-1}$ flow rate in isocratic mode. The minimum detection limit of cyclophosphamide and paclitaxel were 0.5 $\mu g.ml^{-1}$ and 2.0 $\mu g.ml^{-1}$ at retention time of 10.52 min. and 38.59 min. respectively (Larson et al., 2003). The method developed in present study are more reliable to the Larson methods in term of mobile phase use, require less retention time for elution and high sensitivity etc. Sottani et al. (2005) developed HPLC-MS method for the detection for cyclophosphamide and IF from the urine sample health worker. The LOQ of cyclophosphamide and IF in urine was 0.02 and 0.4 $\mu g.L^{-1}$ respectively. In 2008, Sottani et al. developed another RP-HPLC method using ESI-MS for the simultaneous detection of cyclophosphamide, IF, epirubicin, doxorubicin and daunorubicin from human urine sample. The LOQ for cyclophosphamide, IF and epirubicin was 0.2 $\mu g.L^{-1}$, while for daunorubicin and doxorubicin was 150 $ng.L^{-1}$ and 300 $ng.L^{-1}$ respectively (Sottani et al., 2008). Another liquid chromatography (LC-ESI-MS/MS) was developed and validated by Nussbaumer et al. (2010) for the detection of 10 antineoplastic compound (cyclophosphamide, etoposide, IF, epirubicin, doxorubicin, gemcitabine, cytarabine, irinotecan and vincristine) from the hospital waste effluent. The analysis was carried out with acetonitrile, water and formic acid as mobile phase at 0.2 $ml.min^{-1}$ flow rate in isocratic mode. The limit of quantification for these drugs on developed method was in range of concentration from 0.25 to 2 $\mu g.L^{-1}$. Ferrando-Climent et al. (2013) developed UPLC-MS/MS method using SPE for the detection of cyclophosphamide, etoposide and paclitaxel along with seven other antineoplastic compounds from hospital and municipal wastewater samples and validated the same. The sensitivity or LOD of developed method for these compounds was from 0.8 to 24 $ng.L^{-1}$. Here, the mobile phase use for elution of these 10 antineoplastic compounds were 0.1 % formic acid with acetonitrile at 0.4 $ml.min^{-1}$

in gradient mode. This method is highly reliable, sensitive and stable as compared to presently developed methods.

4.3. Morphology and growth of fungi

White rot fungi are basidiomycetes fungi and provide a whitish morphological appearance on their growth. Initially, the collected WRF strains were grown on petri plates for their subculturing. After five to six day of subculture process, the optimum growth was achieved, and the mycelium colonies of fungi were visible on agar petri plates. Among these three fungi, every fungus has their specific morphological appearance as indicated in Fig. 4.7. Furthermore, the growing fungi from petri plates were transferred into selected broth medium for mycelium pellet formation and biodegradation experiment. In broth medium with shaking condition, the morphology of each fungus was different to the static condition. In shaking condition, several spherical beads of mycelium were produced in medium are shown in Fig. 4.8, each fungus has different and specific spherical structure of mycelium pellet in compared to other two fungi. *G. lucidum* grown as spherical bead having hairy structure on their surface, *T. versicolor* grown as spherical beads without any structure on their surface, while *P. chrysosporium* grown as round ball like without any structure on their surface.

Fig. 4.7. Growth of selected WRFs on petri plates (a) *G. lucidum*, (b) *T. versicolor* and (c) *P. chrysosporium*

Fig. 4.8. Growth of selected WRFs under shaking conditions (a) *G. lucidum*, (b) *T. versicolor* and (c) *P. chrysosporium*

4.4. Optimized culture conditions for biomass and enzyme production

The production of biomass and maximum extracellular enzyme activity of *G. lucidum, T. versicolor* and *P. chrysosporium* were estimated under different culture conditions. When the fungi utilized the medium for their growth, consequently the amount of biomass in experimental flasks was also increased in all fungi. This results in increase in the number of cells and their enzyme production. Although, WRFs are highly prominent source to secrete extracellular enzyme, which are highly responsible for the mineralisation of recalcitrant compounds by their oxidation-reduction mechanism. As we found from prior arts, *T. versicolor*, *G. lucidum* and *P. chrysosporium* are also able to produce these enzymes. In present study, we have determined the extracellular enzyme *i.e*. laccase, manganese peroxidase and lignin peroxidase enzyme activity from these three growing fungi culture. The activity of these enzymes was assessed by three different methods or assay that we have discussed in methodology section.

4.4.1. Optimized conditions for biomass and enzyme production by *G. lucidum*

In case of *G. lucidum*, the dry weight of biomass of *G. lucidum* was calculated on 0, 3, 6, 9, 12 and 15 days of experiment. The highest amount of biomass and enzyme activity was estimated at 30 ºC temperature, 5.0 pH and 150 rpm. Here, the initial mycelium biomass size used was 1.505 $g.L^{-1}$ and it was increased to 5.599 ±0.14 $g.L^{-1}$, 8.958 ±1.02 $g.L^{-1}$, 3.871 ±0.042 $g.L^{-1}$, 3.229 ±0.17 $g.L^{-1}$ and 3.084 ±0.011 $g.L^{-1}$ on 3, 6, 9, 12 and 15 days respectively (Fig. 4.9). However, at zero/initial day of experiment, no enzyme activity was observed. The laccase activity of *G. lucidum* was 94.3 ±0.47 $IU.L^{-1}$ on 3rd day, 214.4 ±0.8 $IU.L^{-1}$ on 6th day, 137.5 ±0.61 $IU.L^{-1}$, on 9th day, 76.4 ±0.34 $IU.L^{-1}$ on 12th day and 38.2 ±0.46 $IU.L^{-1}$ observed on 15th day. Manganese peroxidase activity of *G. lucidum* was 31.6 ±1.8 $IU.L^{-1}$ on 3rd day, 64.22 ±0.74 $IU.L^{-1}$ on 6th day, 42.6 ±3.72 $IU.L^{-1}$ on 9th day, 31.96 ±2.22 $IU.L^{-1}$ on 12th day and 18.44 ±0.66 $IU.L^{-1}$ observed on 15th day. Lignin peroxidase activity of *G. lucidum* was 7.78 ±0.68 $IU.L^{-1}$ on 3rd day, 16.3 ±0.47 $IU.L^{-1}$ on 6th day, 6.7 ±0.11 $IU.L^{-1}$ on 9th day, 3.52 ±1.04 $IU.L^{-1}$ on 12th day and 2.36 ±0.24 $IU.L^{-1}$ observed on 15th day. These results indicate, the highest biomass of *G. lucidum* was shown as 8.958 ±1.02 $g.L^{-1}$ on 6th day. Whereas, the maximum laccase, manganese peroxidase and lignin peroxidase activity were shown as 214.4 ±0.8 $IU.L^{-1}$, 64.22 ±0.74 $IU.L^{-1}$ and 16.3 ±0.4 $IU.L^{-1}$ at 6th day respectively (Fig. 4.10).

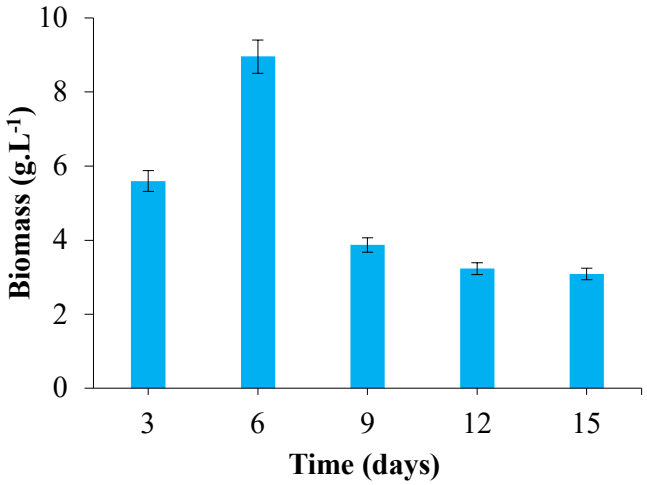


Fig. 4.9. Dry weight biomass analysis under optimized conditions using *G. lucidum*

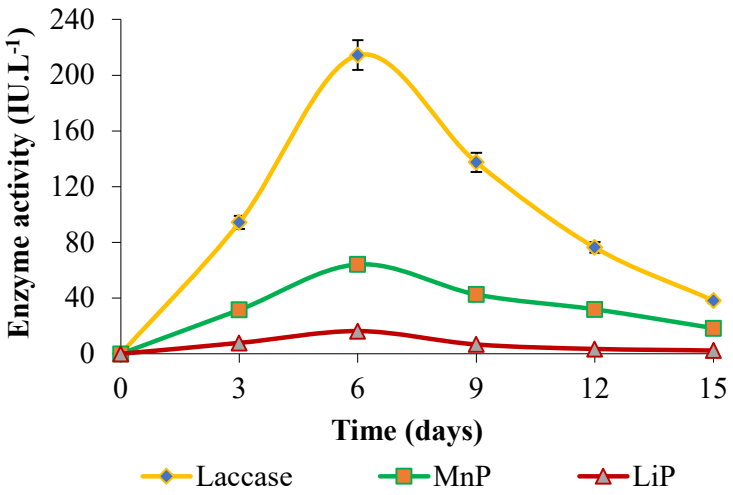


Fig. 4.10. Extracellular enzyme activity analysis under optimized condition using *G. lucidum*

4.4.2. Optimized condition of *T. versicolor* for biomass and enzyme activity

In case of *T. versicolor*, the highest amount of biomass and enzyme activity was estimated at 26 ºC temperature, 4.5 pH and 150 rpm. The initial mycelium biomass size of *T. versicolor* used was 1.552 $g.L^{-1}$ and it was increased to 2.359 ±0.032 $g.L^{-1}$, 4.212 ±0.018 $g.L^{-1}$, 10.84 ±0.812 $g.L^{-1}$, 7.062 ±0.26 $g.L^{-1}$ and 4.032 ±0.025 $g.L^{-1}$ on 3, 6, 9, 12 and 15 days respectively (Fig. 4.11). However, laccase enzyme activity of *T. versicolor* was 70.4 ±5.12 $IU.L^{-1}$ on 3^{rd} day, 148.1 ±5.37 $IU.L^{-1}$ on 6^{th} day, 265.4 ±12.32 $IU.L^{-1}$ on 9^{th} day, 104.8 ±1.44 $IU.L^{-1}$ on 12^{th} day and 56.3 ±4.16 $IU.L^{-1}$ observed on 15^{th} day. The manganese peroxidase activity was estimated as 15.42 ±0.4 $IU.L^{-1}$ on 3^{rd} day, 18.89 ±0.073 $IU.L^{-1}$ on 6^{th} day, 30.32 ±0.96 $IU.L^{-1}$ on 9^{th} day, 14.28 ±0.2 $IU.L^{-1}$ on 12^{th} day and 8.46 ±1.04 $IU.L^{-1}$ observed on 15^{th} day, while lignin peroxidase activity was 12.5 ±1.07 $IU.L^{-1}$ on 3^{rd} day, 22.14 ±1.04 $IU.L^{-1}$ on 6^{th} day, 36.72 ±1.64 $IU.L^{-1}$ on 9^{th} day, 7.5 ±1.35 $IU.L^{-1}$ on 12^{th} day and 2.98 ±0.42 $IU.L^{-1}$ was observed on 15^{th} day. These results indicate, the highest biomass of *T. versicolor* was shown as 10.84 ±0.812 $g.L^{-1}$ on 9^{th} day. Whereas, the maximum laccase, manganese peroxidase and lignin

peroxidase activity were shown as 265.4 ±12.32 IU.L^{-1}, 30.32 ±0.96 IU.L^{-1}and 36.72 ±1.64 IU.L^{-1} at 9th day respectively (Fig. 4.12).

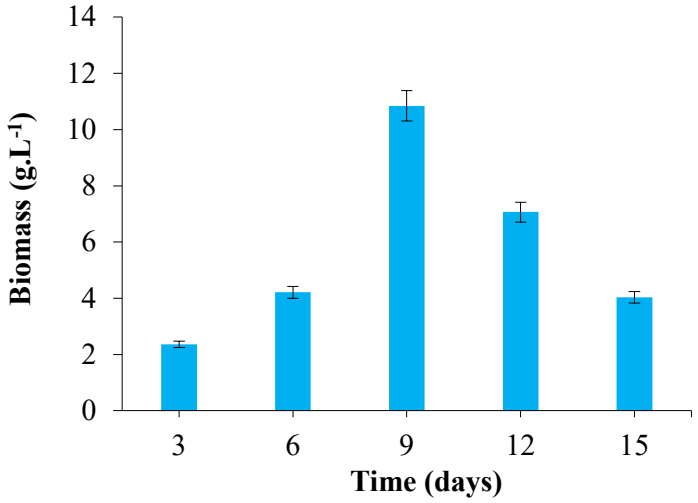


Fig. 4.11. Dry weight biomass analysis under optimized condition using *T. versicolor*

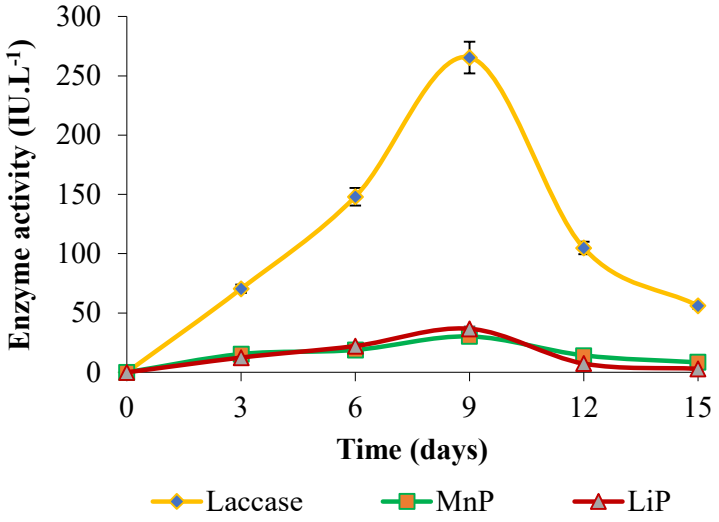


Fig. 4.12. Extracellular enzyme activity analysis under optimized condition using *T. versicolor*

4.4.3. Optimized condition of *P. chrysosporium* for biomass and enzyme activity

In case of *P. chrysosporium*, the highest amount of biomass and enzyme activity were estimated at 30 ºC temperature, 4.5 pH and 180 rpm. The initial mycelium biomass size of

P. chrysosporium used for experiment was 1.496 $g.L^{-1}$ and it was increased to 4.442 ±0.28 $g.L^{-1}$, 9.232 ±0.015 $g.L^{-1}$, 15.148 ±0.423 $g.L^{-1}$, 8.079 ±0.09 $g.L^{-1}$ and 4.728 ±0.024 $g.L^{-1}$ on 3, 6, 9, 12 and 15 days respectively (Fig. 4.13). However, the enzyme activity of *P. chrysosporium,* for laccase was 22.7 ±0.71 $IU.L^{-1}$ on 3rd day, 39.5 ±0.25 $IU.L^{-1}$ on 6th day, 72.1 ±1.97 $IU.L^{-1}$ on 9th day, 18.8 ±1.34 $IU.L^{-1}$ on 12th day and 4.13 ±0.58 $IU.L^{-1}$ observed on 15th day. The manganese peroxidase activity was 72 ±4.15 $IU.L^{-1}$ on 3rd day, 112.8 ±2.24 $IU.L^{-1}$ on 6th day, 186.2 ±5.44 $IU.L^{-1}$ on 9th day, 89.44 ±2.56 $IU.L^{-1}$ on 12th day and 21.6 ±1.02 $IU.L^{-1}$ observed on 15th day, while lignin peroxidase activity was 54.8 ±2.92 $IU.L^{-1}$ on 3rd day, 88.14 ±2.54 $IU.L^{-1}$ on 6th day, 146.3 ±4.81 $IU.L^{-1}$ on 9th day, 61.2 ±3.14 $IU.L^{-1}$ on 12th day and 27.14 ±0.6 $IU.L^{-1}$ was on 15th day. These results indicate, the highest biomass of *P. chrysosporium* was shown as 15.148 ±0.423 $g.L^{-1}$ on 9th day. Whereas, the maximum laccase, manganese peroxidase and lignin peroxidase activity were shown as 72.1 ±1.97 $IU.L^{-1}$, 186.2 ±5.44 $IU.L^{-1}$ and 146.3 ±4.81 $IU.L^{-1}$ at 9th day respectively. In this fungus, the maximum activity was shown for enzyme manganese peroxidase as well for lignin peroxidase as compared to *G. lucidum* and *T. versicolor*, where laccase activity was shown as maximum (Fig. 4.14).

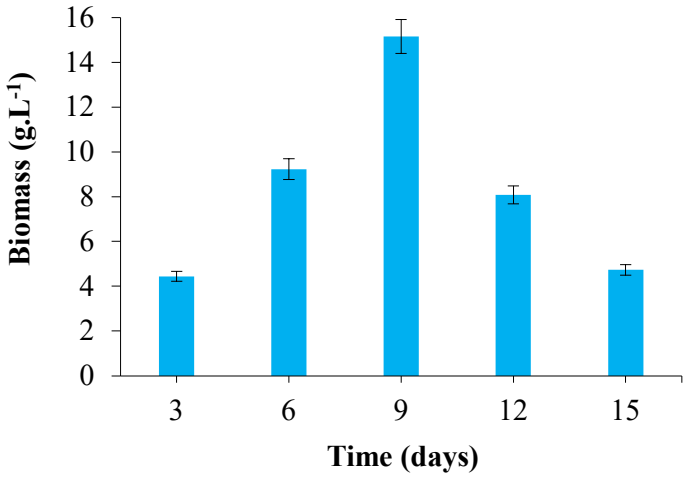


Fig. 4.13. Dry weight biomass analysis under optimized condition using *P. chrysosporium*

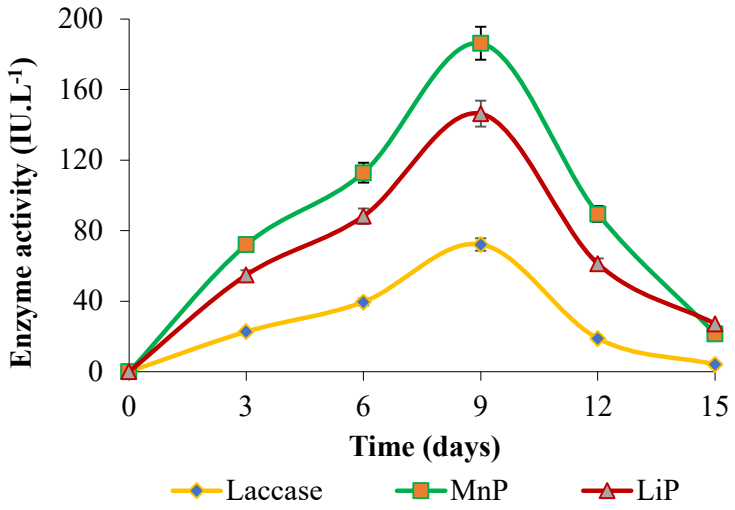


Fig. 4.14. Extracellular enzyme activity analysis under optimized condition using *P. chrysosporium*

In 2008, Wang and their group optimized the culture conditions for maximum extracellular enzyme activity of *P. chrysosporium*. They analysed the activity of two enzyme manganese peroxidase and lignin peroxidase. The fungal culture shown maximum enzyme activity at 30 ºC and 4.5 pH. The maximum activity for enzyme lignin peroxidase and manganese peroxidase was analysed as 541 and 88.5 $IU.L^{-1}$ respectively at 8^{th} day (Wang et al., 2008). Junnarkar et al. (2016), studied the production of lignin peroxidase from *P. chrysosporium*. The maximum enzyme activity was calculated as 33.3 $U.mg^{-1}$ on 6^{th} day at the optimum condition (30 ºC and pH 3.5). Another study performed for the optimization of culture conditions for the production of lignin peroxidase and manganese peroxidase enzymes from *P. chrysosporium* by Couto et al. (2006). According to their analysis, the optimum condition for the maximum production of lignin peroxidase was 34 ºC temperature and 4.2 pH, while for manganese peroxidase was 32 ºC temperature and 4.5 pH. Jayasinghe et al. (2008), studied the optimization of growth condition for the biomass production of *G. lucidum*. According to their analysis, *G. lucidum* was obtained their maximum biomass at 30 ºC and pH 5.0. Another study was performed by Nasreen et al. (2005), for the analysis of biomass production of *G. lucidum*

on different culture conditions. They analysed, the highest biomass production at 25 ºC and pH 5.0 in PDB growth medium as compared to kirk medium and kirk modified medium. Hariharan and Nambisan (2013), optimized the growth condition for the production of extracellular enzyme laccase, lignin peroxidase and manganese peroxidase activity from fungus *G. lucidum*. In their study, they observed maximum enzyme activity at 8th day of inoculation and the enzyme activity for laccase, lignin peroxidase and manganese peroxidase was calculated as 498, 895, 2998 IU.mL^{-1} respectively at 27 ºC and pH 5.0.

Another study performed by Agrawal et al. (2017), for the production and investigation of extracellular enzyme activity from WRFs *G. lucidum*. In this strain, the enzyme activity for laccase, lignin peroxidase and manganese peroxidase was calculated as 1154, 1175 and 15035 IU.L^{-1} respectively. Snajdr and Baldrian (2007), determined the conditions for the production of extracellular enzyme activity of *T. versicolor* fungus. The maximum activity of enzyme laccase and manganese peroxidase was calculated as 370 and 8.7 IU.L^{-1} at 35 ºC. Another study was performed by Qin et al. (2017), for the estimation of laccase activity from *T. versicolor* growing culture. They grow fungus culture at 28 ºC, 6.0 pH and the maximum laccase activity was observed as 1071 IU.L^{-1}on 9th day. These reports indicates that, the enzyme activity shown by the previous studies is higher as compared to the present study, but the culture conditions were different in each finding. The enzyme activity and biomass estimation of *G. lucidum*, *T. versicolor* and *P. chrysosporium* was also estimated by the several researchers during the degradation of different pharmaceutical compounds.

A study investigated the laccase enzyme activity of *T. versicolor* during treatment of two antineoplastic compounds bleomycin and vincristine. The laccase activity of normal fungal culture without antineoplastic compounds was observed as 25 IU.L^{-1}, whereas the activity in case of bleomycin and vincristine treatment culture was reduced to 14 and 20 IU.L^{-1} respectively. In their study, they observed the laccase activity of *T. versicolor* was influenced

by bleomycin and vincristine (Jureczko et al., 2021). In another study, Ferrando-Climent et al. (2015), grown *T. versicolor* at 25 ºC and 4.5 pH for the treatment of antineoplastic compounds azathioprine, cyclophosphamide, etoposide, tamoxifen and paclitaxel. In their study, they estimated the laccase enzyme activity of *T. versicolor* and the maximum laccase activity was calculated as 350 $U.L^{-1}$ on 9th day. Vasiliadou et al. (2016), performed a study for the removal of pharmaceutical compounds using *T. versicolor* and *G. lucidum*. In their study, they monitored the laccase, manganese peroxidase and lignin peroxidase enzyme activity of both fungal cultures. The selected WRFs were grown at 25 ºC, 4.5 pH and 150 rpm. The reported enzyme activity of laccase, manganese peroxidase and lignin peroxidase in *T. versicolor* was 250, 20 and 50 $IU.L^{-1}$ respectively, while in *G. lucidum* it was 20, 40 and 10 $IU.L^{-1}$ respectively.

4.5. Biodegradation of cyclophosphamide, etoposide and paclitaxel

In biodegradation study, the removal efficiency of *P. chrysosporium*, *T. versicolor* and *G. lucidum* fungi for the degradation of cyclophosphamide, etoposide and paclitaxel were estimated. The treatment process involved both degradation and biosorption for the removal of these antineoplastic compounds. The removal efficiency of etoposide was higher as compared to cyclophosphamide and paclitaxel in each case of fungi. Degradation analysis was performed on 3, 6, 9, 12 and 15 days of fungi inoculation (Table 4.6).

In case of *G. lucidum*, the degradation rate of cyclophosphamide was 24.72 % on 3rd day and it was increased to 71.50 % on 6th day. After 6th day, the degradation of cyclophosphamide was very less *i.e*. 0.07 % and total degradation achieved was 71.57 % after 15 days of experiment (Fig. 4.15). The biosorption removal percentage of cyclophosphamide with *G. lucidum* in heat killed control was about 4 %, so the total removal percentage achieved for cyclophosphamide was 75.56 % (Table 4.6). This rate of degradation was significantly different to a study performed by Castellet-Rovira et al. (2018), where *G. lucidum* shows the degradation of

cyclophosphamide less than 40 %, although laccase activity was also present in sample *i.e.* 100 $U.L^{-1}$. The total removal percentage of etoposide by *G. lucidum* was >99 %. After 3 days, the degradation of etoposide was 58.27 % and it was increased to 98.49 % at 6th day of treatment process (Fig. 4.15). Further, it was slightly increased to 98.94 % at 9th day. The biosorption rate of etoposide with *G. lucidum* was 0.75 %, that was quite low as compared to cyclophosphamide (Table 4.6). The total removal rate of paclitaxel by *G. lucidum* was about 21 %. Here, the biodegradation percentage for paclitaxel was achieved 8. 23 % on 3rd day of immobilization and it was increased to 19.54 % on 6th day. After 6th day the degradation rate was slightly increased *i.e.* 0.63 % and further no more degradation was achieved for paclitaxel (Fig. 4.15). The soprtion removal percentage of paclitaxel with *G. lucidum* was about 1.67 %, while maximum degradation percentage was 20.17 % (Table 4.6). Here, these results shows that, the *G. lucidum* achieved their maximum degradation for cyclophosphamide, etoposide and paclitaxel at 6th day but in etoposide it was quite high as compared to cyclophosphamide and paclitaxel.

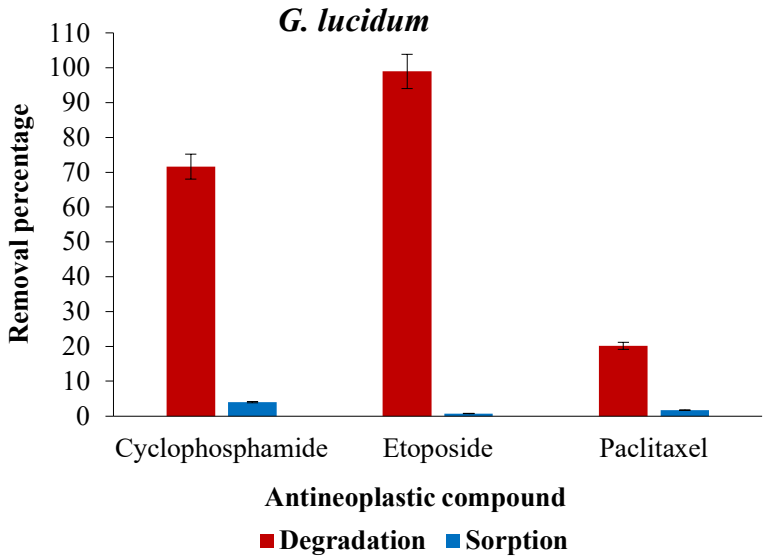


Fig 4.15. Total removal efficiency of *G. lucidum* for cyclophosphamide, etoposide and paclitaxel

The removal of cyclophosphamide, etoposide and paclitaxel by *T. versicolor* was highly different in term of degradation and biosorption percentage. The total removal rate of cyclophosphamide was negligible *i.e*. 1.47 % along with biosorption rate. After 15 days of experiment, the degradation rate was 1.04 % and biosorption rate was 0.43 %**.** The degradation rate of etoposide was quite high as compared to cyclophosphamide. The total removal rate of etoposide was about 97 %, while maximum biodegradation rate achieved was 79.93 % (Fig. 4.16). After 3 days, the degradation of etoposide with *T. versicolor* was observed and it was about 31.47 %, further the rate was increased up to 79.82 % on 6^{th} day. After 6 days very less concentration of etoposide was reduced *i.e*. 0.11 % and it was almost negligible. Further, no more removal was observed in the collected sample were analysed on LC-MS. These results indicates that, the maximum degradation for etoposide was achieved by *T. versicolor* on 6^{th} day. The biosorption rate of etoposide with *T. versicolor* was also very high. It was about 18.02 % indicated in the LC-MS data of collected samples from biosorption control flask (Table 4.6). The paclitaxel degradation was start after 3 days and at 6^{th} day the degradation rate of paclitaxel with *T. versicolor* was about 3.12 % and it was increased to 5.91 % and 6.07 % on 9^{th} and 15^{th} day respectively (Fig. 4.16). After 9 days, very minor concentration of paclitaxel was degraded. The biosorption rate of paclitaxel with *T. versicolor* was 2.36 %. So, the total removal rate was about 8.43 % after 15 days of treatment process (Fig. 4.16). The biodegradation of cyclophosphamide and etoposide was studied by Ferrando-Climent et al. (2015). According to their study, the removal of cyclophosphamide was about 1 % and they reported no removal of cyclophosphamide by *T. versicolor*, even after laccase activity was showing in sample, while the etoposide was totally removed from the sample. Another study was also conducted for the removal of cyclophosphamide by Castellet-Rovira et al. (2018) and they investigate that the cyclophosphamide was removing but the removal rate was below 40 % of their initial concentration. Ferrando-Climent et al. (2015) also performed a study for removal of paclitaxel

from hospital wastewater by the help of *T. versicolor* but they were not founded paclitaxel presence in the sample and the degradation study of paclitaxel was not confirmed.

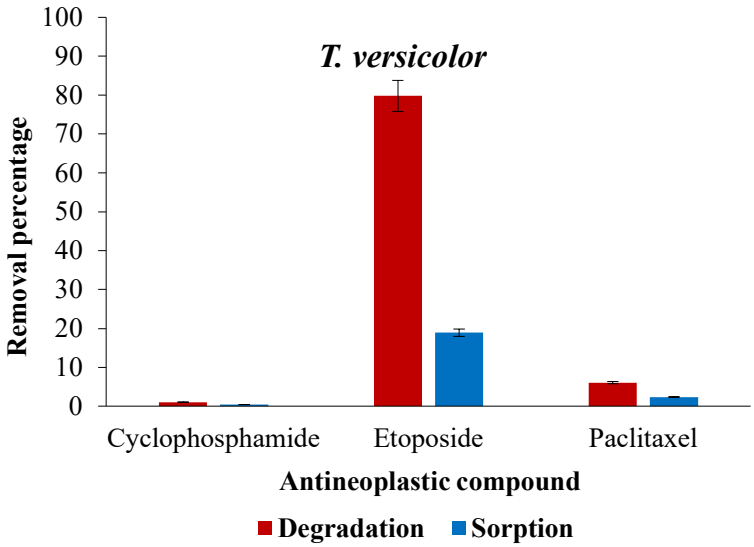


Fig. 4.16. Total removal efficiency of *T. versicolor* for cyclophosphamide, etoposide and paclitaxel

In case of *P. chrysosporium*, the degradation of cyclophosphamide was also negligible. No biodegradation of cyclophosphamide was achieved during biodegradation with *P. chrysosporium* after the 15 days of experiment. However, some concentration of cyclophosphamide from the samples was reduced by the biosorption process. The biosorption rate of cyclophosphamide by *P. chrysosporium* was about 23.77 % (Fig. 4.17). The degradation of etoposide was quite different, it was analysed about 32.64 % on 6th day and it was increased to 44.71 %, 69. 57% and 76.85 % on 9, 12 and 15 days respectively. The maximum degradation rate was 76.85 % achieved after 15 days of degradation experiment, while total removal percentage was about 98 % (Table 4.6). The biosorption rate of etoposide in *P. chrysosporium* was about 21.25 %, it was high as like cyclophosphamide biosorption rate (Fig. 4.17). This fungus also shown degradation for paclitaxel, but it was very low**.** The degradation of paclitaxel with *P. chrysosporium* was quite different in comparison to other two compounds. Here, no

degradation was analysed on 3rd day, while after 3rd day the degradation was start and it was increased to 2.63 % on 6th day, 4.37 % on 9th day and 4.91 % on 15th day respectively. The total biodegradation percentage of paclitaxel with this fungus was 4.91 % and the biosorption percentage was 1.42 %, so the total removal percentage of paclitaxel was 6.33 % (Fig. 4.17). These results indicates that, *P. chrysosporium* have ability only for the removal of etoposide, while degradation of some percentage of paclitaxel was also achieved but their rate was very low. In cyclophosphamide, the removal was only shown through biosorption process, there was no biodegradation, but it shows the highest biosorption removal efficiency among all the selected fungi.

Table 4.6. Biodegradation of selected antineoplastic compounds with *G. lucidum*, *T. versicolor* and *P. chrysosporium*

Antineoplastic compounds (Initial concentration 300 µg.L^{-1})		**Total degradation rate (%) (Average of triplicate)**				
		3 days	6 days	9 days	12 days	15 days
Ganoderma lucidum	Cyclophosphamide	24.72	71.50	71.57	71.57	71.57
	Etoposide	58.27	98.49	98.94	98.94	98.94
	Paclitaxel	8.23	19.54	20.17	20.17	20.17
Trametes versicolor	Cyclophosphamide	0	0	1.04	1.04	1.04
	Etoposide	31.47	79.82	79.93	79.93	79.93
	Paclitaxel	0	3.12	5.91	6.07	6.07
Phanerochaete Chrysosporium	Cyclophosphamide	0	0	0	0	0
	Etoposide	18.79	32.64	44.71	69	76.85
	Paclitaxel	0	2.63	4.37	4.91	4.91

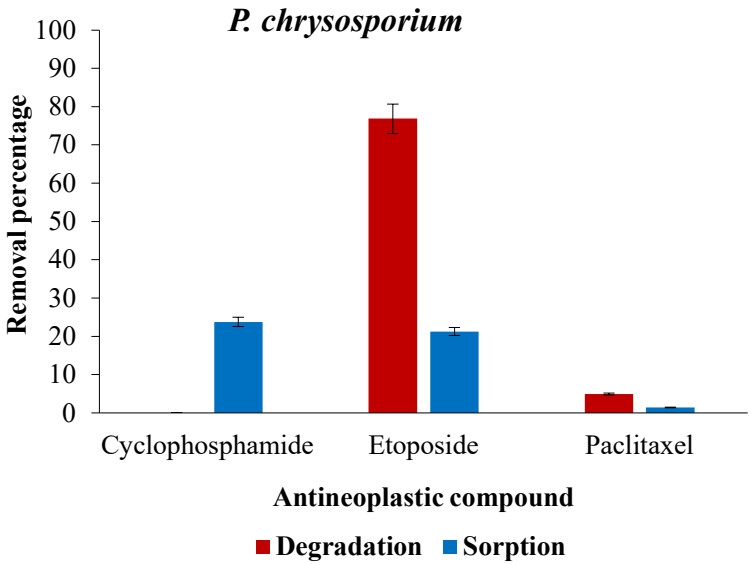


Fig. 4.17. Total removal efficiency of *P. chrysosporium* for cyclophosphamide, etoposide and paclitaxel

Table 4.7. Total efficiency of *G. lucidum*, *T. versicolor* and *P. chrysosporium* for the removal of cyclophosphamide and etoposide

WRFs	Antineoplastic compound	Total removal rate (In percentage)	Biosorption rate (In percentage)	Biodegradation rate (In percentage)
G. lucidum	Cyclophosphamide	75.56 %	3.99 %	71.57 %
	Etoposide	99.69 %	0.75 %	98.94 %
	Paclitaxel	21.84 %	1.67 %	20.17 %
T. versicolor	Cyclophosphamide	1.47 %	0.43 %	1.04 %
	Etoposide	98.75 %	18.93 %	79.82 %
	Paclitaxel	8.43 %	2.36 %	6.07 %
P. chrysosporium	Cyclophosphamide	23.77 %	23.77 %	No degradation
	Etoposide	98.1 %	21.25	76.85 %
	Paclitaxel	6.33 %	1.42 %	4.91 %

The present result indicates that, among these three WRFs, *G. lucidum* have highest potential for the removal of cyclophosphamide, etoposide and paclitaxel, while *T. versicolor* and *P. chrysosporium* shows the removal efficiency only for etoposide and very less for paclitaxel but not for cyclophosphamide (Table 4.6). The removal of paclitaxel was shown by all the fungi but the removal rate was very low and in case of *T. versicolor* and P. *chrysosporium*, their removal was less than 10 %, it was quite insignificant removal.

Apart from cyclophosphamide, etoposide and paclitaxel, several other antineoplastic compounds also have been removed from these WRFs by the various researchers (Table 2.5). *G. lucidum* and *T. versicolor* were used for the elimination of IF and iopromide also. The *T. versicolor* removed iopromide about 47 % of their initial concentration (174.4 $\mu g.L^{-1}$) and out of which about 85 % removal was observed through degradation and 15 % was through biosorption. The removal of IF was only about 25 % from their initial concentration (39.1 $\mu g.L^{-1}$) and from which only less than 10 % was removed through degradation. *G. lucidum* removed IF and iopromide less than 40 % of their initial concentration and in both cases, the biodegradation was less than 60 % of their total removal (Castellet-Rovira et al., 2018). Another study performed by Ferrando-Climent et al. (2015) by the use of *T. versicolor* for removal of tamoxifen, azathioprine and IF. In their study, the removal of tamoxifen and IF was performed in both hospital wastewater and synthetic sample, where removal of azathioprine was only performed in hospital wastewater sample. The initial concentration in hospital wastewater sample of tamoxifen, IF and azathioprine were 0.0445 $\mu g.L^{-1}$, 0.077 $\mu g.L^{-1}$ and 0.055 $\mu g.L^{-1}$ respectively, while in synthetic sample initial concentration of tamoxifen and IF were 0.0003 $mg.ml^{-1}$ and 0.01 $mg.ml^{-1}$ respectively. The tamoxifen was removed about 48 % in hospital wastewater sample, while in synthetic sample it was removed about 92 % after 1 hour and 99 % after 9 days of experiment, in which about 82 % after 24 hours and 94 % after 9 day was removed through biosorption process. The removal of IF was

only achieved in real hospital wastewater sample and removal rate was 61 %, whereas no removal was observed for IF in synthetic sample. The azathioprine was totally removed (100 %) from real wastewater sample.

Jureczko et al. (2021) studied biodegradation of vincristine and bleomycin by *T. versicolor*, *F. fomentarius, H. fasciculare, P. nidulans* and *P. ostreatus*. They used 10 $mg.L^{-1}$ as initial concentration of vincristine and bleomycin. The total removal efficiency of *T. versicolor, F. fomenatrius* and *H. fasciculare* for vincristine was achieved 94 % after 4 days and 97 % after 14 days, while in *P. oestrus* the efficiency was 52.5 %. However, no removal of vincristine was observed in *P. niulans* mediated treatment. The biosorption rate of vincristine with *T. versicolor*, *F. fomentarius* and *H. fasciculare* was analysed as 14 % to 29 % of total removal (97 %) of their concentration. The removal of bleomycin was observed only with *T. versicolor* and *H. fasciculare*. The removal rate of bleomycin analysed with *T. versicolor* was 36 % after 9 days and 64 % after 14 days, while in *H. fasciculare* was 58.5 % observed after 14 days of experiment. However, no removal of bleomycin was observed with *F. fomenatrius*, *P. oestrus* and *P. niulans*. The biosorption rate of bleomycin with *T. versicolor* was about negligible, while in *H. fasciculare* it was about 29 % observed. *P. chrysosporium* was used to remove diclofenac (Zhang and Geißen, 2010), tetracycline and oxytetracycline (Wen et al., 2010). Lucas et al. (2018) performed a study on elimination of four antibiotics through biosorption process of three different WRFs. The WRFs strain used for removal of diclofenac, carbamazepine, venlafaxine and iopromide were *T. versicolor, G. lucidum* and *I. lacteus*. They found highest biosorption rate of diclofenac in case of all fungi *i.e*. 42 %, 34 % and 26 % observed in case of *T. versicolor*, *G. lucidum* and *I. lacteus* respectively, while total biodegradation was 54 %, 64 % and 71 % observed in case of *T. versicolor*, *G. lucidum* and *I. lacteus* respectively. The biosorption rate was also shown for rest three antibiotics, but it was very less as compared to diclofenac. For carbamazepine, the biosorption was 8 %, 13 % and

5 %, while biodegradation was 50 %, 23 % and 57 % observed in case of *T. versicolor*, *G. lucidum* and *I. lacteus* respectively. In iopromide, the biosorption rate was 8 %, 18 % and 5 %, while biodegradation was 39 %, 13 % and 22 % observed in case of *T. versicolor*, *G. lucidum* and *I. lacteus* respectively. The biosorption rate of venlafaxine was 6 %, 11 % and 6 %, and biodegradation was 49 %, 19 % and 9 % observed in case of *T. versicolor*, *G. lucidum* and *I. lacteus* respectively. Another study was also investigated by Jureczko et al. (2021) for analysis of biosorption efficiency of two antineoplastic compounds vincristine and bleomycin on five WRFs strains. The WRF strain used for this investigation were *F. fomentarius, H. fasciculare, P. nidulans, P. ostreatus,* and *T. versicolor*. They used alive or dead biomass for the analysis of biosorption efficiency and the found variation in efficiency for both dead and alive biomass in case of each fungus. In case of alive biomass, the biosorption efficiency of bleomycin was 23 %, 15 %, 11 %, 6 % and 0 % with *T. versicolor, P. ostreatus, P. nidulans, H. fasciculare* and *F. fomentarius* respectively, while vincristine shown 14 %, 16 %, 20 %, 7 % and 7 % efficiency with *T. versicolor, P. ostreatus, P. nidulans, H. fasciculare* and *F. fomentarius* respectively. In case of dead biomass, the biosorption efficiency of bleomycin was 35 %, 23 %, 0 %, 29 % and 38 % with *T. versicolor, P. ostreatus, P. nidulans, H. fasciculare* and *F. fomentarius* respectively, while vincristine shown 17 %, 14 %, 8.6 %, 16 % and 13 % efficiency with *T. versicolor, P. ostreatus, P. nidulans, H. fasciculare* and *F. fomentarius* respectively. This study reports the highest biosorption efficiency of bleomycin in alive biomass with *T. versicolor*, while in dead biomass was shown with *F. fomentarius*. The highest biosorption efficiency of vincristine was shown with *P. nidulans*, while in dead biomass was shown with *T. versicolor*.

4.6. Negative effect of antineoplastic compounds on fungi

The dry weight biomass of control and experimental flask was calculated to analyse the negative effect of cyclophosphamide, etoposide and paclitaxel on growth of fungi. There was

no effect of these compounds observed on all three fungi. They were produced the same amount of biomass size with each antineoplastic compounds that was produced during the optimization of biomass production before the treatment process as shown in Fig. 4.9, 4.11 and 4.13. A study performed by Jureczko et al. (2021), for the biomass estimation of five WRFs (*F. fomentarius, H. fasciculare, P. nidulans, P. ostreatus and T. versicolor*) during treatment of antineoplastic compounds bleomycin and vincristine. In their study they investigated that, the effect of bleomycin and vincristine was different in each case of fungal culture. In some fungi, the biomass was increased as compared to the control biomass growth, while in some case the biomass or growth was inhibited as compared to the control biomass growth. The WRFs *P. nidulans* was not able to remove bleomycin and vincristine but their biomass was increased to the control. However, vincristine was not exhibiting negative effect on the growth of all fungi. So, they concluded that, each pharmaceutical compound has a particular influence on the growth of each fungal growth and their degradation ability does not depend on the biomass inhibition.

4.7. Estimation of pH and reducing sugar

During the biodegradation study, simultaneously the pH value and glucose concentration were estimated. Here, we have analysed the pH value and estimated the glucose concentration of experimental flask on every day of sampling. On the basis of the variation in these parameters, we provide a prediction towards relation of pH and glucose concentration on rate of degradation.

4.7.1. pH

Initially, the pH of growth medium was adjusted according to the best growing condition of fungi for the degradation of pharmaceutical. The pH of *G. lucidum* was adjusted 5.0, while for *T. versicolor* and *P. chrysosporium* it was 4.5 adjusted at initial day of experiment. The pH of

all the experimental and control flask were checked or analysed at every movement of the sampling *i.e.* 0, 3, 6, 9, 12 and 15 days. The pH value of *G. lucidum* was observed 5.0, 3.72 ±0.08, 4.39 ±0.09, 6.74 ±0.1, 6.92 ±0.06 and 6.30 ±0.13 at 0, 3, 6, 9, 12 and 15 days respectively (Fig. 4.18 (a)). The pH value of *T. versicolor* was observed 4.5, 4.46 ±0.07, 5.02 ±0.1, 4.52 ±0.14, 5.87 ±0.08 and 6.5 ±0.1 at 0, 3, 6, 9, 12 and 15 days respectively (Fig. 4.18 (b)), whereas the pH value *P. chrysosporium* was observed 4.5, 3.76 ±0.8, 3.87 ±0.12, 6.04 ±0.1, 4.98 ±0.12 and 5.86 ±0.14 on 0, 3, 6, 9, 12 and 15 days respectively during the degradation experiment (Fig. 4.18 (c)).

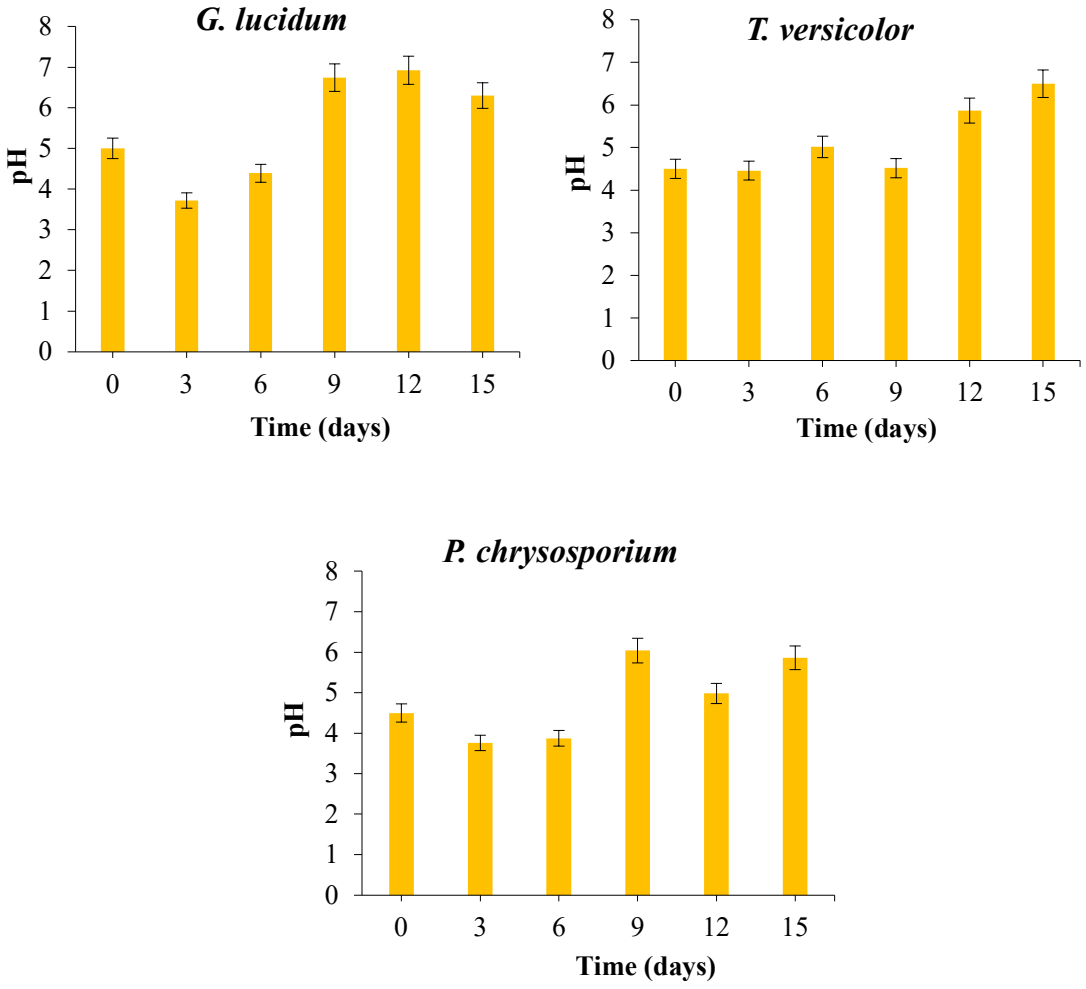
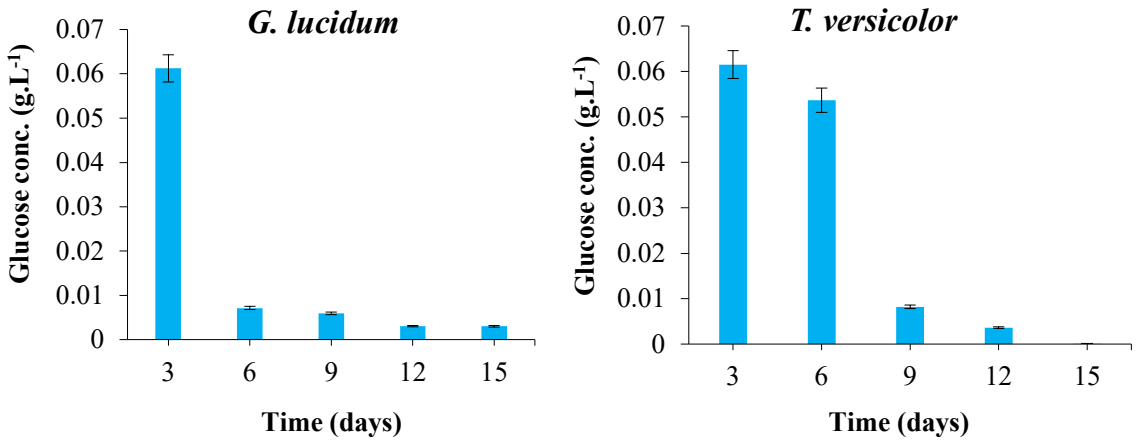


Fig. 4.18. pH analysis during degradation of antineoplastic compounds using (a) *G. lucidum* (b) *T. versicolor* (c) *P. chrysosporium*

4.7.2. Glucose estimation

During degradation, in growth medium the glucose was used as carbon source for fungi. The initial glucose concentration used at zero day was 10 $g.L^{-1}$ in each flask. On every day of sampling along with other analysis, glucose concentration was also estimated in term of reducing sugar. In case of *G. lucidum*, it was calculated as 0.06121 ±0.0084 $g.L^{-1}$, 0.00714 ±0.0002 $g.L^{-1}$, 0.005962 ±0.00028 $g.L^{-1}$, 0.00306 ±0.00003 $g.L^{-1}$ and 0.00302 ±0.000022 $g.L^{-1}$ on 3, 6, 9, 12 and 15 days respectively (Fig. 4.19 (a)). In *T. versicolor*, the glucose concentration was calculated as 0.0615 ±0.00274 $g.L^{-1}$, 0.5367 ±0.00098 $g.L^{-1}$, 0.0822 ±0.00038 $g.L^{-1}$, 0.00362 ±0.00052 $g.L^{-1}$ and 0.0012 ±0.00007 $g.L^{-1}$ on 3, 6, 9, 12 and 15 days respectively (Fig. 4.19 (b)). The glucose concentration in *P. chrysosporium* was calculated as 0.00551 ±0.0004 $g.L^{-1}$, 0.00393 ±0.00036 $g.L^{-1}$, 0.0022 ±0.00019 $g.L^{-1}$, 0.00016 ±0.00002 $g.L^{-1}$ and 0.00011 ±0.00001 $g.L^{-1}$ on 3, 6, 9, 12 and 15 days respectively (Fig. 4.19 (c)). These results indicate, the maximum concentration of glucose was utilized by the fungi at their initial stage of degradation. Among these three fungi, the highest glucose concentration was utilized by *P. chrysosporium* from start to end of the experiment.



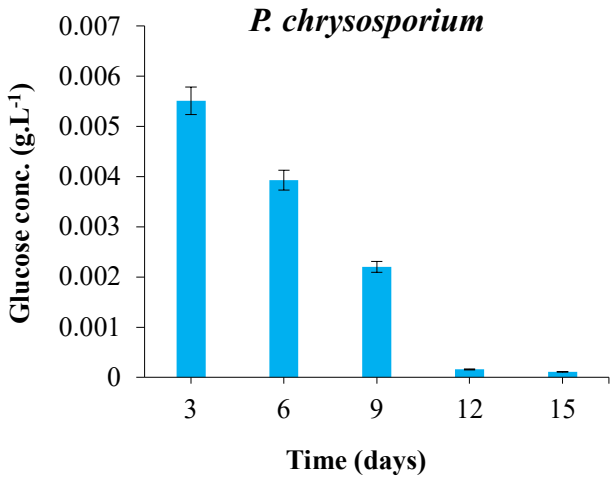


Fig. 4.19. Pattern of glucose utilization by (a) *G. lucidum* (b) *T. versicolor* and (c) *P. chrysosporium* during degradation of antineoplastic compounds

4.7.3. Time-course degradation relationship with pH and glucose

In G. lucidum treatment flasks, the rate of degradation was achieved for all three compounds but the percentage was different. The highest degradation of etoposide, cyclophosphamide and paclitaxel achieved was 98 %, 71 % and 20 % respectively. Although, the variation in pH value of *G. lucidum* in each antineoplastic case was similar. Initially, the pH value was decreased from 5.0 to 3.72 ±0.08 and further, it was continuously increased till 15 days. The *G. lucidum* achieve their maximum efficiency for the degradation of cyclophosphamide, etoposide and paclitaxel at pH 4.39 ±0.09. However, the maximum utilization of glucose concentration was achieved only at the end of 3rd day and after that only very less amount was present (Fig. 4.20). So, the effect of glucose concentration on degradation rate is not confirm during this treatment process.

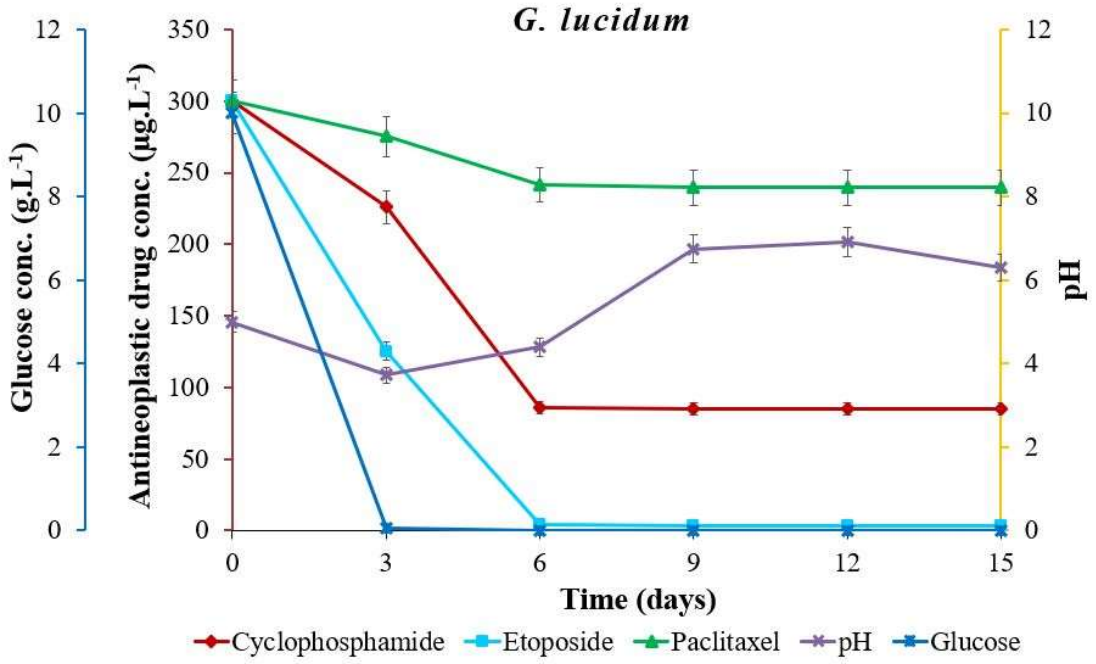


Fig. 4.20. Time course of cyclophosphamide, etoposide, paclitaxel degradation along with pH and glucose by *G. lucidum*

In *T. versicolor* the highest activity for the degradation of cyclophosphamide, etoposide and paclitaxel was highly different to *G. lucidum*. In this fungus, the significant degradation was achieved only for etoposide. However, very minute degradation of paclitaxel was also achieved but no biodegradation was existing for cyclophosphamide in this study. The initial pH of *T. versicolor* mediated treatment flask was 4.5 and it was increased to 5.02 ±0.1 on 6th day. The maximum degradation rate of etoposide was also observed at 6th day. These results indicate, the maximum activity of *T. versicolor* was shown at pH 5.02 ±0.1 for degradation of etoposide. The removal of paclitaxel was shown after 15 days *i.e.* 6.07 % at pH 5.87 ±0.08 but it was not much significant. The initial glucose this treatment was also high. Here, the maximum amount of glucose was utilized at end of 3 days, but after 3 days the glucose utilization reduced and become very less (Fig. 4.21). In this treatment also, the effect of glucose concentration on rate of degradation is not confirmed.

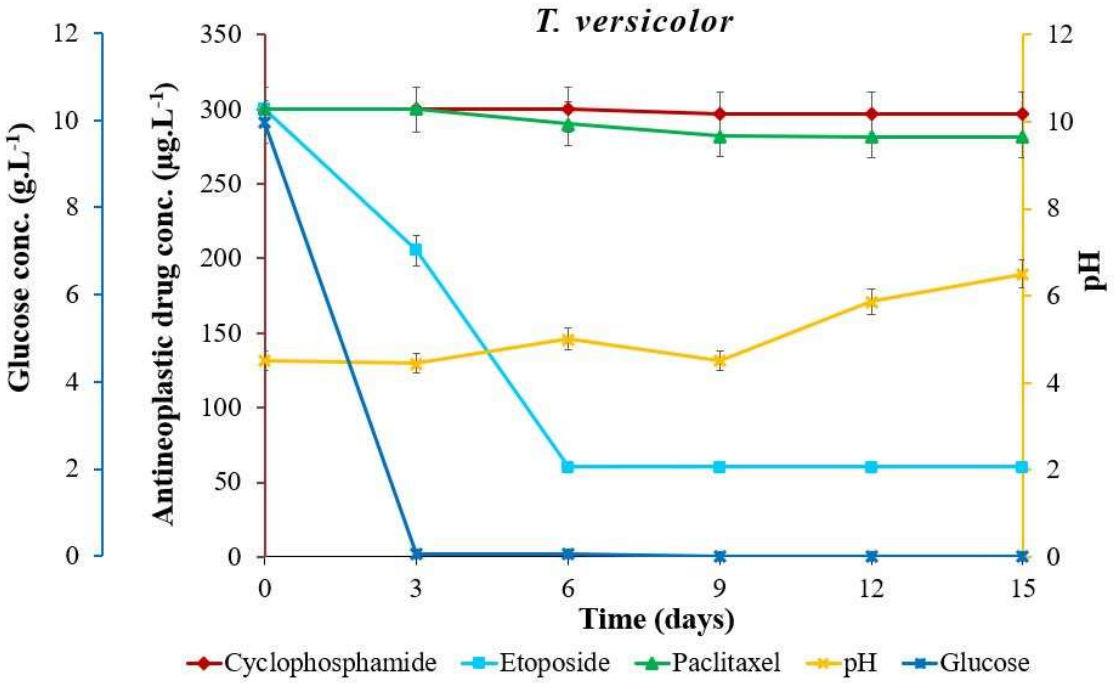


Fig. 4.21. Time course of cyclophosphamide, etoposide, paclitaxel degradation along with pH and glucose by *T. versicolor*

In *P. chrysosporium* the degradation was only shown for etoposide. Some activity was also shown for paclitaxel *i.e*. 4.91 % after 15 days but it not significant. Here, the initial value of growing treatment flask was 4.5 and it was reduced to 3.76 ±0.8 and 3.87 ±0.12 on 3rd and 6th day but after this the pH value was increased. The highest degradation activity of etoposide was shown at 15th day and the pH of experimental flask at 15th day was 5.86 ±0.14. These results indicates that the maximum degradation activity of *P. chrysosporium* was achieved at pH 5.86. Whereas the glucose utilization in *P. chrysosporium* was maximum at 3rd day and after this very minute concentration of glucose was present in the culture (Fig. 4.22). So, same as like other two fungi, the relation of glucose on degradation activity is not confirmed during this treatment process. Among all three WRFs, the maximum and fast glucose was utilized by the *P. chrysosporium*.

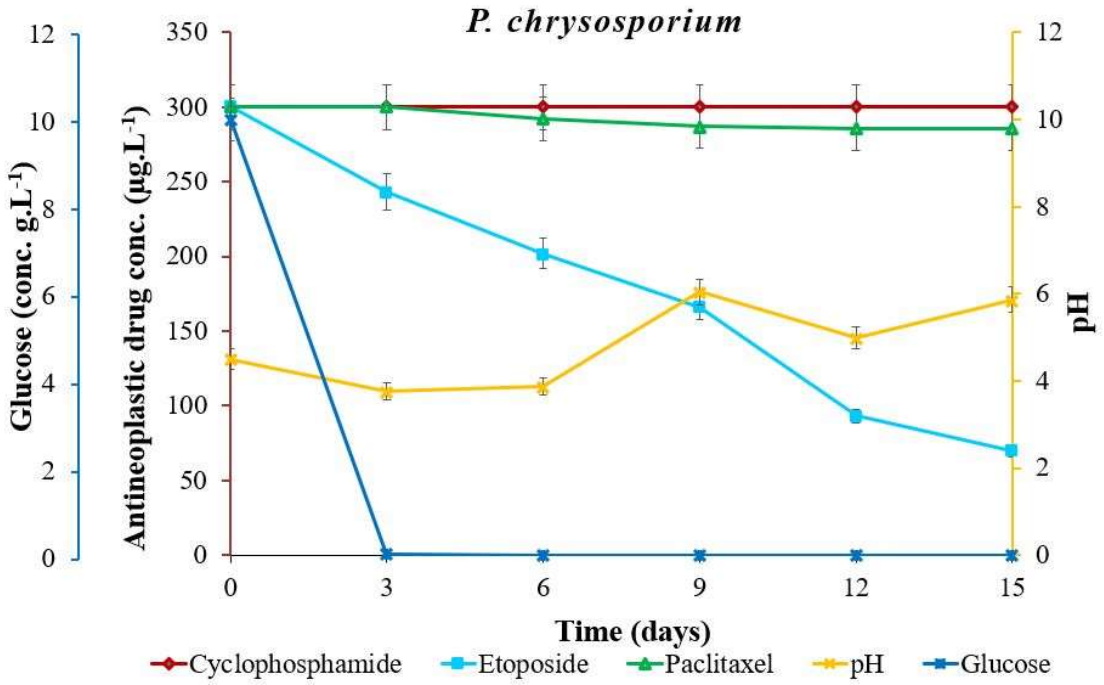


Fig. 4.22. Time course of cyclophosphamide, etoposide, paclitaxel degradation along with pH and glucose by *P. chrysosporium*

According to these findings, the maximum removal efficiency of *G. lucidum* for cyclophosphamide, etoposide and paclitaxel achieved at pH 4.39 ±0.09. In *T. versicolor* it was demonstrated at pH 5.02±0.1 for etoposide. The initial pH of the *P. chrysosporium* treatment flask was 4.5, which was reduced to 3.76±0.8 and 3.87±0.12 on the third and sixth days, respectively. The highest etoposide removal efficiency was observed on the 15th day, and the pH of the experimental flask on the 15th day was 5.86±0.14. So, the maximum removal efficiency of *P. chrysosporium* was achieved at a pH of 5.86. Only at the end of the third day the maximum utilization of glucose was observed, and after that, only a very small amount was present in the sample. However, because the degradation rate was highest in *G. lucidum*, *T. versicolor* on sixth day and in *P. chrysosporium* continuous degradation till 15th day, the current study cannot co-relate the degradation efficiency with the glucose concentration utilization. Ferrando-Climent et al. (2015), also performed investigation on time-course

biodegradation of antineoplastic compounds by *T. versicolor* with simultaneous glucose consumption analysis. Similar to the present study, glucose consumption was utilized in the initial days or second day of the degradation experiment. Another study conducted by Castellet-Rovira et al. (2018), they analysed the glucose concentration during the treatment of antineoplastic compounds with six WRFs (*T. versicolor*, *I. lacteus*, *S. rugosoannulata*, *G. lucidum*, *A. erebia*, *G. luteofolius*). In their study, they measured glucose concentration only at the start and end day of experiment. In the end of experiment, they reported no glucose concentration was present in the sample of each fungal culture and the pH remained stable *i.e.* 4.5.

4.8. Degradation kinetics and kinetics model study

In present work during the degradation, the biodegradation kinetics of antineoplastic compounds by these three WRFs was also evaluated. There is degradation rate depends on the concentration of antineoplastic compounds, so herein only pseudo-first-order reaction was applied for kinetics study. Zero-order and Second-order reaction were not eligible for this degradation study because in these reactions the rate of reaction is not depends or directly proportional to the substrate or antineoplastic compound concentration.

In *G. Lucidum* treatment, all three antineoplastic compounds had followed the pseudo-first-order kinetics with r^2 values 0.8774, 0.902 and 0.8774 cyclophosphamide, etoposide and paclitaxel respectively. Among these three compounds, the maximum degradation was achieved for etoposide on 3rd day and the degradation rate constant '*k*' value was 0.5453 day^{-1}, whereas the rate constant of cyclophosphamide and paclitaxel was 0.1564 day^{-1} and 0.0285 day^{-1} respectively. The theoretical half-time ($t_{1/2}$) of drug degradation value calculated for etoposide was 1.27113 day, while for cyclophosphamide it was 4.43189 days. The half-time of paclitaxel was highly different to etoposide and paclitaxel. Here, the value of paclitaxel was

24.31 days as it was degraded only 20.17 % of the initial concentration (300 µg.L^{-1}) till 15 days (Fig. 4.23).

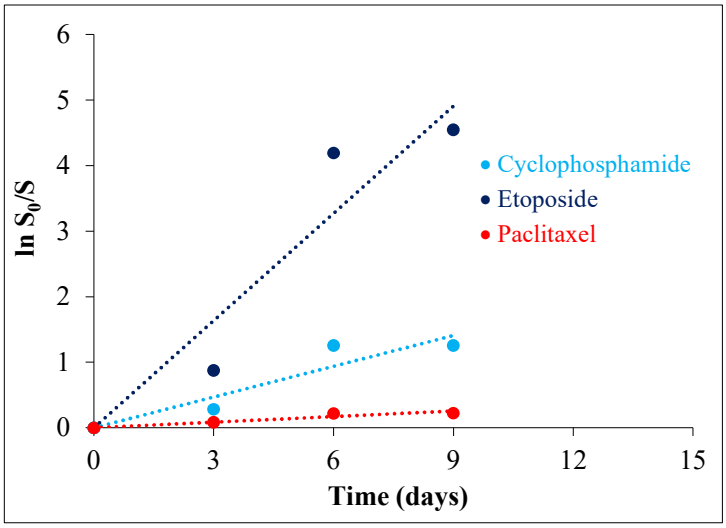


Fig. 4.23. Pseudo-first-order kinetics model for degradation of cyclophosphamide, etoposide and paclitaxel with *G. lucidum*

In *T. versicolor* treatment experiment, the degradation of etoposide was quite comparable with *G. lucidum*, while degradation of cyclophosphamide and paclitaxel was highly different. In this treatment, the etoposide was degraded up to 79.82 % at 6th day but for cyclophosphamide and paclitaxel the rate was 1.04 %, 6.07 % till 15 days. Etoposide and paclitaxel followed pseudo-first-order degradation path with r^2 values 0.8981 and 0.8911 respectively, but cyclophosphamide degradation path showed r^2 value only 0.7455 for the same kinetics. The degradation rate constant was maximum for etoposide *i.e.* 0.2386 day^{-1}, while for cyclophosphamide and paclitaxel the '*k*' value was 0.0008 day^{-1} and 0.0055 day^{-1}. These low kinetics constant of cyclophosphamide and paclitaxel was achieved because both the compounds were nearly unaffected by this WRF. The calculated half-time value of the initial concentration (300 µg.L^{-1}) for cyclophosphamide was 866.434 days (more than two years) and

lowest for etoposide *i.e*. 2.90506 days with this fungus. Half-time calculated for paclitaxel was 126.027 days (Fig. 4.24).

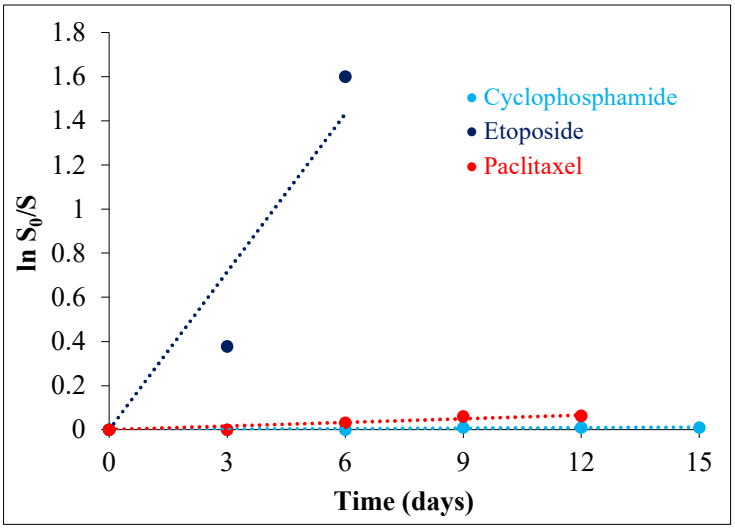


Fig. 4.24. Pseudo-first-order kinetics model for degradation of cyclophosphamide, etoposide and paclitaxel with *T. versicolor*

In *P. chrysosporium* degradation, the cyclophosphamide was not degraded till 15 days of experiment, but the degradation kinetics of etoposide investigated successfully with pseudo-first-order degradation kinetics. Here, 76.85 % degradation of etoposide was achieved with *P. chrysosporium* within 15 days of experiment. The r^2 value of etoposide was calculated as 0.9425 and degradation rate constant '*k*' achieved was 0.0896 day^{-1} with half time of degradation 7.73602 days, when initial drug concentration was 300 $\mu g.L^{-1}$. Paclitaxel was degraded only 4.91 % after 15 days and the kinetics investigated suggested pseudo-first-order degradation path for this removal with r^2 value 0.9104. The degradation rate constant and half-time value for paclitaxel in this experiment were 0.0043 day^{-1} and 161.197 days respectively (Fig. 4.25).

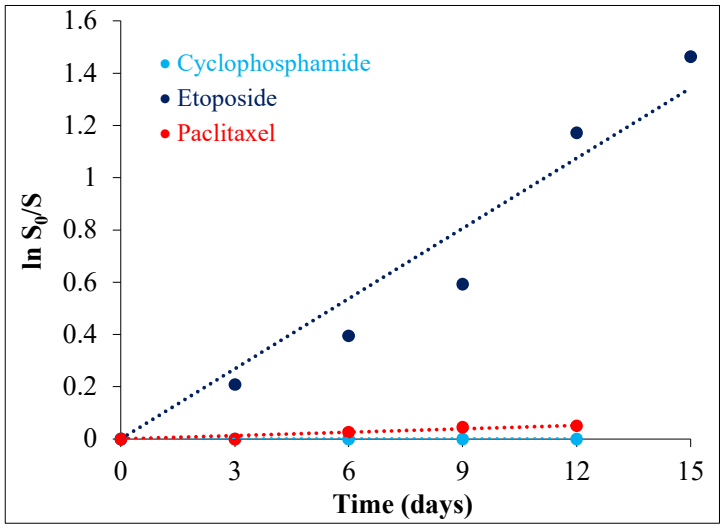


Fig. 4.25. Pseudo-first-order kinetics model for degradation of cyclophosphamide, etoposide and paclitaxel with *P. chrysosporium*

Among these three WRFs, the maximum degradation rate constant was calculated in treatment of etoposide with *G. lucidum i.e.* 0.5453 day^{-1} whereas, the lowest degradation rate constant was shown by *T. versicolor* for treatment of cyclophosphamide *i.e.* 0.0008 day^{-1}. The degradation kinetics of allethrin by fungus *Fusarium proliferatum* was studied by Bhatt et al. (2020). They investigated that the fungal strain was completely removed allethrin from the sample in 5 days. The fungal culture followed the pseudo-first-order degradation kinetics. The $t_{1/2}$ was calculated as 26.05 h with rate constant (k) value 0.0193 h^{-1}. Another study was performed by Bankole et al. (2020), on the investigation of biodegradation kinetics of three pharmaceutical compounds *i.e.* diclofenac, celecoxib and ibuprofen by using WRFs *G. lucidum* and *Laetiporus suplhureus*. They followed both pseudo-first-order kinetics model and pseudo-second-order kinetics model for the degradation kinetics analysis. In pseudo-first-order kinetics, the $t_{1/2}$ of diclofenac, celecoxib and ibuprofen were calculated as 0.99, 0.91 and 0.98 days respectively with 'k' constant value 0.29, 6.72 and 4.2 day^{-1} respectively. A study investigated by Zhang et al. (2019), for the degradation of sulfadiazine degradation with *P. chrysosporium.* The degradation study followed the pseudo-order-reaction kinetics for the

analysis. They performed degradation at different pH range *i.e.* 3.7, 4.7, 5.7 and 6.7. The degradation '*k*' constant values on above pH for sulfadiazine were calculated as 0.04 d^{-1}, 0.43 d^{-1}, 0.46 d^{-1} and 0.33 d^{-1} respectively. These studies indicates that the degradation rate constant of a pharmaceutical compound different or vary in treatment with each fungus and only pseudo-first-order kinetics model is applicable for degradation kinetics analysis of pharmaceutical compounds with WRF.

4.9. Cyto-toxicity analysis of native and degraded products of antineoplastic compounds

4.9.1. Cyto-toxicity of native antineoplastic compounds

In this study, the MTT assay was performed by using Raw 264.7 cell line to evaluate the cytotoxicity of cyclophosphamide, etoposide and paclitaxel which are widely used for cancer treatment and showing presence in water bodies. The MTT assay was applied to detect (Lojk et al., 2020) cell viability and the percentage of cytotoxicity on Raw 264.7 cells with different doses of cyclophosphamide, etoposide and paclitaxel *i.e.* 10, 25, 50, 100, 150, 200 and 250 $\mu g.ml^{-1}$. After 48 hours of antineoplastic compound treatment, the viability of cells was determined and the results of cytotoxicity for above mentioned antineoplastic compounds described in Table 4.8. The cytotoxicity percentage of cyclophosphamide on Raw 264.7 cell at concentration 10, 25, 50, 100, 150, 200 and 250 $\mu g.ml^{-1}$ were 17.48 %, 26.17 %, 34.22 %, 41.96 %, 50.07 %, 61.69 % and 69.58 % respectively. In case of etoposide the percentage of cytotoxicity on Raw 264.7 cell at an exposure concentration 10, 25, 50, 100, 150, 200 and 250 $\mu g.ml^{-1}$ were 42.99 %, 52.22 %, 63.35 %, 74.86 %, 80.23 %, 87.29 % and 92.01 % respectively. While the cytotoxicity of paclitaxel on Raw 264.7 cell at concentration 10, 25, 50, 100, 150, 200 and 250 $\mu g.ml^{-1}$ were 31.07 %, 36.49 %, 44.08 %, 59.57 %, 75.25 %, 84.9 % and 88.85 % respectively. At 48 hours of antineoplastic compounds treatment indicated the consistent decrease in cell viability of Raw 264.7 cells with an increase in the concentration of

cyclophosphamide, etoposide and paclitaxel. These three antineoplastic compounds were significantly inhibiting proliferation in Raw 264.7 cell line by exhibiting cytotoxic effects. The IC_{50} value for cyclophosphamide was 145.44 ±2.67 $\mu g.ml^{-1}$, etoposide was 15.40 ±5.92 $\mu g.ml^{-1}$ and paclitaxel was 69.76 ±4.23 $\mu g.ml^{-1}$ on 10, 25, 50, 100, 150, 200 and 250 $\mu g.ml^{-1}$ concentrations (Table 4.8). IC_{50} of etoposide was the lowest as compared to cyclophosphamide and paclitaxel as indicated in Fig. 4.26 and Table 4.7. So, among these three antineoplastic compounds, etoposide indicating the highest cytotoxicity on Raw 264.7 cells because if the IC_{50} value of the compound is lower, the compounds will be more cytotoxic and it was determined by designing a dose-response curve. The cytotoxic response of different concentrations of these three antineoplastic compounds on Raw 264.7 cell line is shown in Fig. 4.26 (a-c). The inhibitory concentration value showing half-maximal effective concentration obtained from the relative potency of selected antineoplastic compounds and the dose-response curve.

In prior studies, AT and CT of cyclophosphamide, etoposide and paclitaxel were analysed on *V. fischeri, B. calyciflorus, C. dubia, D. rerio, L. minor, D. magna* and *T. platyurus* (Table 4.9). The toxicity was determined in the form of EC_{50}/LC_{50}. The values of EC_{50}/LC_{50} for cyclophosphamide on *V. fischeri* were >100 $\mu g.ml^{-1}$ (acute) and 1396 $\mu g.ml^{-1}$ (chronic), *B. calyciflorus* were 1924 $\mu g.ml^{-1}$ (acute) and 89.84 $\mu g.ml^{-1}$ (chronic), *C. dubia* were 986.6 $\mu g.ml^{-1}$ (acute) and 58.03 $\mu g.ml^{-1}$ (chronic), *T. platyurus* was 1396 $\mu g.ml^{-1}$ (acute) and *L. minor* was >100 $\mu g.ml^{-1}$ (chronic) (Lutterbeck et al., 2016; Białk-Bielińska et al., 2017; Russo et al., 2018). In etoposide, value of toxicity on *B. calyciflorus* were >120 $\mu g.ml^{-1}$ (acute) and 3.7 $\mu g.ml^{-1}$ (chronic), *C. dubia* was 0.204 $\mu g.ml^{-1}$ (chronic), *D. rerio* was >100 $\mu g.ml^{-1}$ (acute), *T. platyurus* was 74.85 $\mu g.ml^{-1}$ (acute) and *D. magna* was 0.239 $\mu g.ml^{-1}$ (chronic) (Kovács et al., 2016; Parrella et al., 2014a). In paclitaxel, toxicity was determined on *D. magna* was >0.074 $\mu g.ml^{-1}$ (acute) (CDER, 1996).

Table 4.8. Determination of cytotoxicity and inhibitory concentration of cyclophosphamide, etoposide and paclitaxel

Antineoplastic compound	Concentration dose (µg.ml^{-1})	Cell cytotoxicity percentage	IC_{50} (µg.ml^{-1})
Cyclophosphamide	10	17.48	145.44 ±2.67
	25	26.17	
	50	34.22	
	100	41.96	
	150	50.07	
	200	62.69	
	250	69.58	
Paclitaxel	10	31.07	69.76 ±4.23
	25	36.49	
	50	44.08	
	100	59.57	
	150	75.25	
	200	84.9	
	250	88.85	
Etoposide	10	42.99	15.40 ±5.92
	25	52.22	
	50	63.35	
	100	74.86	
	150	80.23	
	200	87.29	
	250	92.01	

It shows that, the cytotoxic effect of cyclophosphamide and etoposide is lower as well as higher on Raw 264.7 cells as compared to previous studies but in the case of paclitaxel toxicity is lower as compared to prior studies. Researchers determined the AT and CT of these antineoplastic compounds on different animal model and microorganism such as bacteria, algae etc. by estimating different parameters such as luminescence, growth inhibition mortality, immobilisation and reproduction inhibition is given in Table 4.9 but the cytotoxic effect of cyclophosphamide, etoposide and paclitaxel on Raw 264.7 cell is not yet analysed.

In view of the above result, for cyclophosphamide the percentage of cell cytotoxicity was 17.48 % minimum at an exposure concentration 10 $\mu g.ml^{-1}$ and 69.58 % maximum at 250 $\mu g.ml^{-1}$, while the 50 % of cell death occurred at 145.44 ±1.67 $\mu g.ml^{-1}$. The linear regression value for cyclophosphamide was 0.9519 (Fig. 4.26 (a)). The etoposide was showing higher cell cytotoxicity that was 42.99 % minimum at concentration 10 $\mu g.ml^{-1}$ and 92.01% maximum at concentration 250 $\mu g.ml^{-1}$, while the 50 percent cell death occurred at 5.40 ±0.94 $\mu g.ml^{-1}$. The linear regression value for etoposide was 0.9185 (Fig. 4.26 (b)). In present study, the cytotoxicity percentage of paclitaxel on Raw 264.7 cells was 31.07 % minimum at concentration 10 $\mu g.ml^{-1}$ and 88.85 % maximum at concentration 250 $\mu g.ml^{-1}$, while 50 % of cell death after the treatment of paclitaxel was at concentration 69.76 ±4.23 $\mu g.ml^{-1}$. The linear regression value for paclitaxel was 0.9717 (Fig. 4.26 (c)). It reveals that etoposide is more toxic for Raw 264.7 cells as compared to cyclophosphamide and paclitaxel.

This is the first study performed, in which MTT assay was used to detect cytotoxic effect of above antineoplastic compounds on the immune cell line. The test was based on the standard colorimetric assay by the estimation of cell proliferation or cell growth. The present study showed cyclophosphamide, etoposide and paclitaxel are cytotoxic for Raw 264.7 cells while their cytotoxicity is varying accordingly.

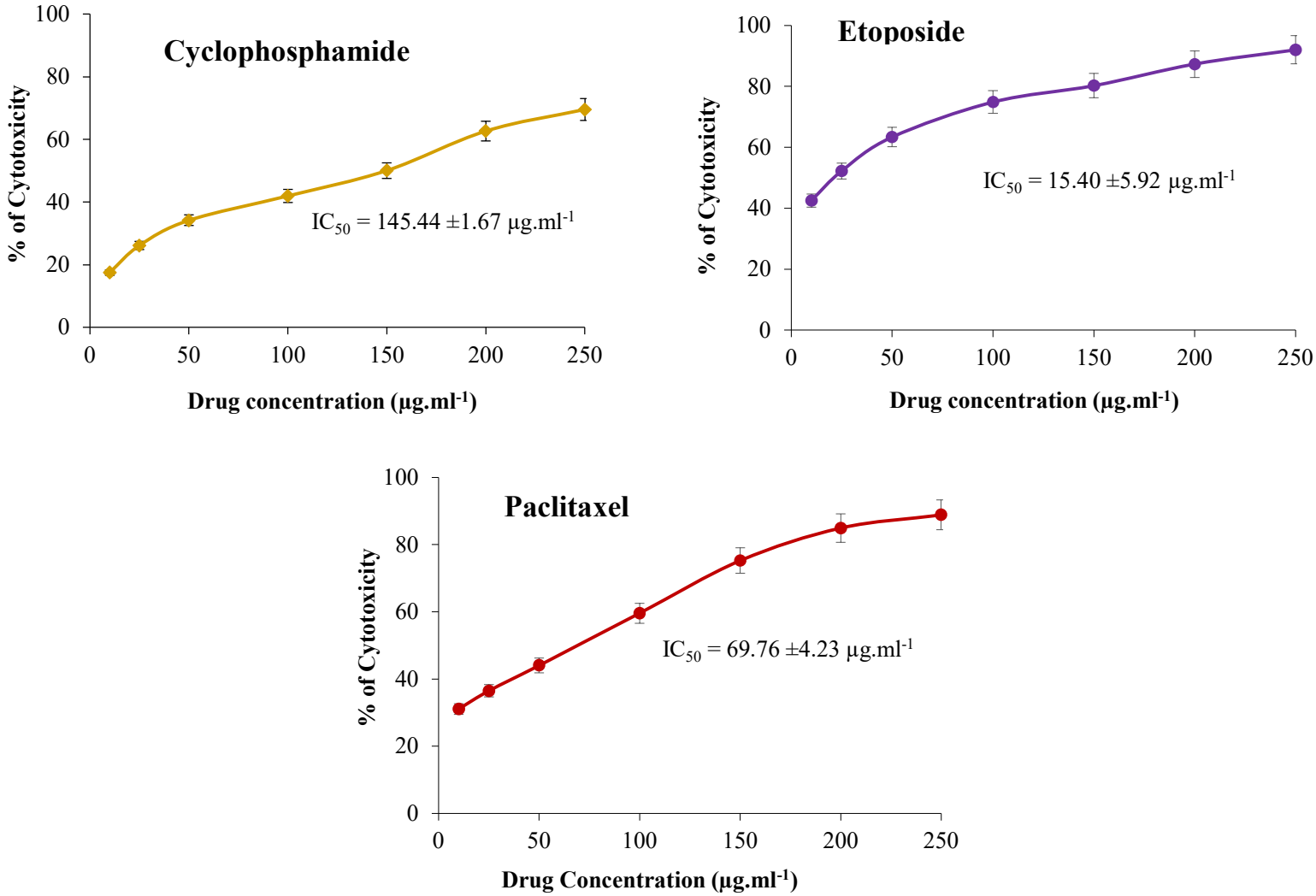


Fig 4.26. Dose-response curve of a) cyclophosphamide b) etoposide and c) paclitaxel

Table 4.9. Toxicity data of cyclophosphamide, paclitaxel and etoposide on different organism (Heath et al., 2020; Russo et al., 2020)

Antineoplastic compounds	Toxicity type	Organism	Toxicity (EC_{50}/LC_{50}) $mg.ml^{-1}$	References
Cyclophosphamide	Acute	*Vibrio fischeri*	>0.1	(Białk-Bielińska et al., 2017)
		Brachionus calyciflorus	1.924 (1.210–3.036)	(Russo et al., 2018)
		Ceriodaphnia dubia	0.986 (0.765–1.272)	(Russo et al., 2018)
		Daphnia magna	>1.0	(Zounková et al., 2007)
			>0.1	(Białk-Bielińska et al., 2017)
		Thamnocephalus platyurus	1.396 (1.304–1.494)	(Russo et al., 2018)
	Chronic	*Vibrio fischeri*	1.396 (1.304–1.494	(Lutterbeck et al., 2016)
		Synechococcus leopoliensis	>0.120	(Česen et al., 2016a)
			>0.320	(Česen et al., 2016a)
			>0.1	(Białk-Bielińska et al., 2017)

			>0.2	(Russo et al., 2018)
		Brachionus calyciflorus	0.089 (0.067–0.119)	(Russo et al., 2018)
		Ceriodaphnia dubia	0.058 (0.037–0.089)	(Russo et al., 2018)
		Daphnia magna	>0.1	(Grung et al., 2008)
		Lamna minor	>0.1	(Białk-Bielińska et al., 2017)
Paclitaxel	Acute	*Daphnia magna*	>.00074	(CDER, 1996)
Etoposide	Acute	*Brachionus calyciflorus*	>0.12	(Parrella et al., 2014b)
		Ceriodaphnia dubia	16 % at 0.120	(Parrella et al., 2014b)
		Daphnia magna	25 % at 0.120	(Parrella et al., 2014b)
			0.030 (0.016–0.040)	(Zounková et al., 2007)
		Thamnocephalus platyurus	0.074 (0.056–0.099)	(Parrella et al., 2014b)
		Danio rerio	>0.1	(Kovács et al., 2016)
	Chronic	*Pseudokirchneriella subcapitata*	0.250 (0.120–0.460)	(Zounková et al., 2007)

Brachionus calyciflorus	0.0037 (0.0027–0.0053)	(Parrella et al., 2014b)
Ceriodaphnia dubia	0.002 (0.0015–0.0025)	(Parrella et al., 2014b)
Daphnia magna	0.0023 (0.0018–0.0029)	(Parrella et al., 2014b)

The cytotoxic effect shown by antineoplastic compounds in MTT assay cause cell growth inhibition in Raw 264.7 cells which may be occur due to DNA strand breakage or topoisomerase activity. So, the above finding indicates that these antineoplastic compounds considered as potential cytotoxic agent and cause damage of the immune system or body of an organism when come in contact with these compounds.

4.9.2. Cytotoxicity of transformed/degraded products

The cytotoxicity of by-products generated during biodegradation of cyclophosphamide, etoposide and paclitaxel by *G. lucidum*, *T. versicolor* and *P. chrysosporium* was determined by cell viability assay. In this study, MTT assay was performed to investigate the cell viability of Raw 264.7 cells when treated with TPs of cyclophosphamide etoposide and paclitaxel. Toxicity of TPs was analysed of sample collected on 3, 6, 9, 12 and 15 day of degradation experiment. As we discussed in section 4.4, the cyclophosphamide was removed only by *G. lucidum*, so except from this fungus, in rest two fungi mediated treatment flask, the cyclophosphamide was remained same or present in their native form from the beginning to end of the degradation experiment. The toxicity level of cyclophosphamide drug control was 7.68 % and medium

control was 0.44 % for Raw 264.7 cells was observed. In *G. lucidum* mediated treatment, the cell cytotoxicity percentage of cyclophosphamide was 1.42 %, 2.38 %, 2.01 %, 2.41 % and 2.31 % measured on 3, 6, 9, 12 and 15 days respectively. In *T. versicolor* it was 6.72 %, 6.65 %, 6.87 %, 7.06 % and 7.03 measured on 3, 6, 9, 12 and 15 days respectively, while in *P. chrysosporium* it was 7.31 %, 6.87 %, 6.22 %, 6.39 % and 6.17 % analysed on 3, 6, 9, 12 and 15 days respectively. These results of cyclophosphamide indicates that, the cyclophosphamide was remained same in case of *T. versicolor* and *P. chrysosporium* and their toxicity level Raw 264.7 cells was almost similar in both fungi treated samples from beginning to end. There was not much difference reported in the toxicity and this may be due to non-degradation of cyclophosphamide by these fungi. While the toxicity of *G. lucidum* treated sample indicates difference in cell cytotoxicity level in compared to these fungi. Here, the toxicity level of TPs on Raw 264.7 cells was decreased as compared to their native compound toxicity level. At initial degradation, the toxicity level of TPs was less *i.e.* 1.42 % and it was increased to 2.38 % on 6^{th} day. Further, no more variation in toxicity was observed or it was observed almost equal on all day of sampling (Fig. 4.27).

These results analysed that the toxicity level of TPs of cyclophosphamide was reduced after the degradation with this fungus. When the cytotoxicity of native form of cyclophosphamide on Raw 264.7 cell line was analysed, we determined the cell cytotoxicity in the form of $IC_{50.}$ The IC_{50} value of cyclophosphamide was determined between 10 to 250 $\mu g.ml^{-1}$ and the 50 % of cell were affected on concentration of 145.44 ±2.67 $\mu g.ml^{-1}$ (Table 4.8). The current study of toxicity of cyclophosphamide defined by the cell cytotoxicity percentage of treated sample. Here, the concentration of antineoplastic compound in sample was less as compared to previous study and cell cytotoxicity percentage also varying but this may be due to the exposure of concentration level of cyclophosphamide on Raw 264.7 cells. (Ferrando-Climent et al., 2015) performed an investigation on toxicity evaluation of *T. versicolor* mediated degraded sample

of cyclophosphamide on *V. fischeri* but due to non-biodegradation of cyclophosphamide by *T. versicolor*, the toxicity of treated sample was same from beginning to end of experiment.

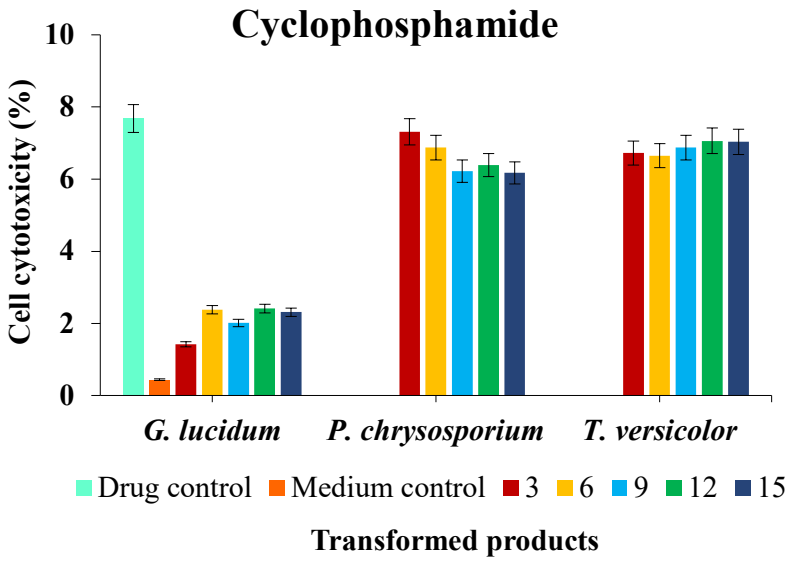


Fig. 4.27. *In-vitro* cytotoxicity of TPs of cyclophosphamide on Raw 264.7 cells during biodegradation with *G. lucidum*, *P. chrysosporium* and *T. versicolor*

In case of etoposide, each TPs were able to generate toxicity on Raw 264.7 cells but their cell cytotoxicity percentage was varying in each fungi treatment sample according to TPs generated. The toxicity level of etoposide drug control was 18.22 % and medium control was 0.45 % for Raw 264.7 cells was observed. The cytotoxicity percentage of *G. lucidum* mediated TPs of etoposide was observed 0.83 % on 3rd day and it was increased to 1.46 %, 1.43 %, 1.57 % and 1.48 % on 6, 9, 12 and 15 days respectively. Here, the toxicity of etoposide TPs increased after 3 days and further it was analysed almost similar on 6, 9, 12 and 15 days. This may be due to the maximum degradation was achieved on 6 day and after that no new TPs were form and the toxicity was remained same. The cell cytotoxicity percentage of by *P. chrysosporium* mediated TPs of etoposide was observed 4.11 % on 3rd day and it was continuously increased to 4.95 % on 6th day, 7.95 % on 9th day, 8.03 % on 12th day respectively. After this, the toxicity

level of TPs was reduced to 7.02 on 15th day. These results of toxicity provide the highest cell cytotoxicity of TPs of etoposide on Raw 264.7 cells during this treatment process. Here, the toxicity of TPs was already higher at the initial degradation, but it was continuously increased after 3 days. Moreover, the toxicity level was reduced at the end of experiment but still it was higher to their initial rate. The toxicity percentage of *T. versicolor* mediated TPs was analysed 2.19 on 3rd day. After 3 days, the cytotoxicity level was increased from 2.19 % to 2.46 %, further it was reduced to 2.06 % and again it was slightly increased 2.74 %, 2.68 % on 12 and 15 days respectively (Fig. 4.28). These results indicating that, the initial formed TPs were less toxic for Raw 264.7 cells but during biodegradation some more toxic TPs were generated as compared to initial TPs of etoposide produced during this process.

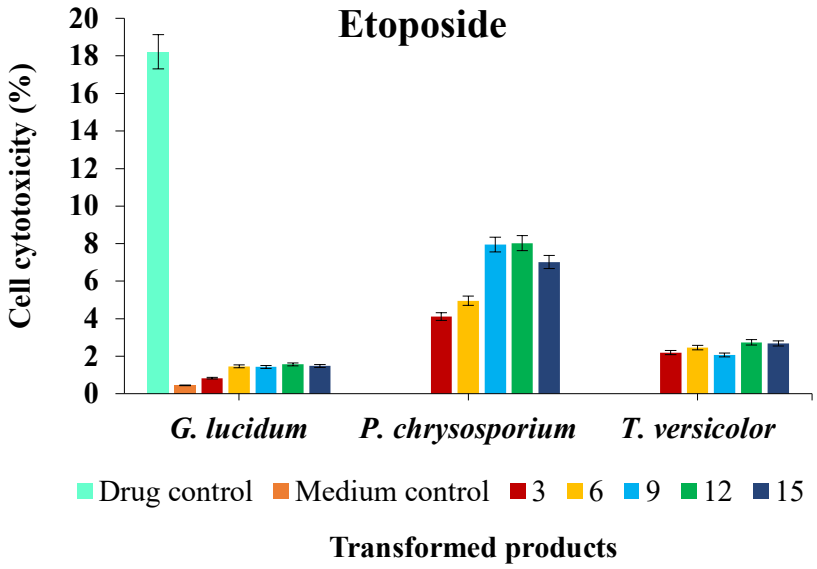


Fig. 4.28. *In-vitro* cytotoxicity of TPs of etoposide and on Raw 264.7 cells during biodegradation with *G. lucidum*, *P. chrysosporium* and *T. versicolor*

In case of *P. chrysosporium*, the cell cytotoxicity of etoposide was higher *i.e.* 8.03 % analyse from TPs produced during treatment with other two fungi. This indicate that, the *P. chrysosporium* can remove etoposide from the aquatic sample but after degradation it can

generate more toxic by-products of etoposide as compared to *G. lucidum* and *T. versicolor* mediated TPs. The cell cytotoxicity of *G. lucidum* mediated TPs of etoposide exhibited less toxicity in comparison to other two fungi mediated TPs. During the parent compounds toxicity analysis, we determined that the etoposide was highly cytotoxic for Raw 264.7 cells and their toxicity estimated in the form of IC_{50}. The value of etoposide for Raw cells was 15.40 ±5.92 $\mu g.ml^{-1}$ when cells were treated between a range of 10 to 250 $\mu g.ml^{-1}$ (Table 4.8).

This indicated that, the native form of etoposide was quite higher toxic for these cells. However, after the degradation with fungi, their TPs were also able to generate cell cytotoxicity and toxicity of level of each TPs was different. Several studies for the investigation of toxicity of cyclophosphamide and etoposide were also performed by the researchers but they carried out their studies as AT and CT on microorganism such as *D. magna*, *V. fischeri*, *B. calciflorus*, *C. dubia*, *L. minor* etc. in the form EC_{50}/LC_{50} (Table 4.9). Their toxicity range varied and it was depending on the drug concentration taken for the exposure of microorganism (Česen et al., 2016b; Lutterbeck et al., 2016; Białk-Bielińska et al., 2017 Russo et al., 2018).

In case of paclitaxel, the degradation rate was extremely low. Here, only in *G. lucidum* some concentration of paclitaxel was reduced, while in case of other fungi, the degradation of paclitaxel was less than 7 %. Therefore, the cell cytotoxicity of paclitaxel and TPs formed during treatment with fungi was analysed on Raw 264.7 cells. The toxicity level of paclitaxel drug control was 11.33 % and medium control was 0.45 % for Raw 264.7 cells observed. In *G. lucidum*, it was estimated 10.31 % on 3rd day. At this movement of sampling, the degradation rate was very low, so mainly the toxicity of native form was present but after this when the degradation rate was increased, toxicity level was slightly decreased continuously up to end of experiment. The cell cytotoxicity percentage on 6, 9, 12 and 15 days was estimated 9.42 %, 8.42 %, 8.38 % and 8.03 % respectively. These result shows that the toxicity of paclitaxel was

reduced, when the degradation rate was increased but due to low degradation it was not much reduced to their initial level. In *T. versicolor*, the cell cytotoxicity percentage was 11.39 % on 3rd day and after that the toxicity level reduced. The cytotoxicity of 6, 9, 12 and 15 days was analysed 10.14 %, 10.07 %, 10.02 % and 10.04 % respectively. The toxicity percentage on 3rd day was high because up to this time, no degradation of paclitaxel was start and only native form of paclitaxel was present. Here, after 3 days, the toxicity level was reduced but it was high to *G. lucidum* mediated samples and this may be due to low degradation rate of paclitaxel with *T. versicolor*. The cell cytotoxicity percentage of *P. chrysosporium* mediated sample of paclitaxel was observed 11.27 % on 3rd day, while after 3 days the value of toxicity was reduced. It was analysed 10.41 %, 10.29 %, 10.16 % and 10.11 % on 6, 9, 12 and 15 days respectively (Fig. 4.29).

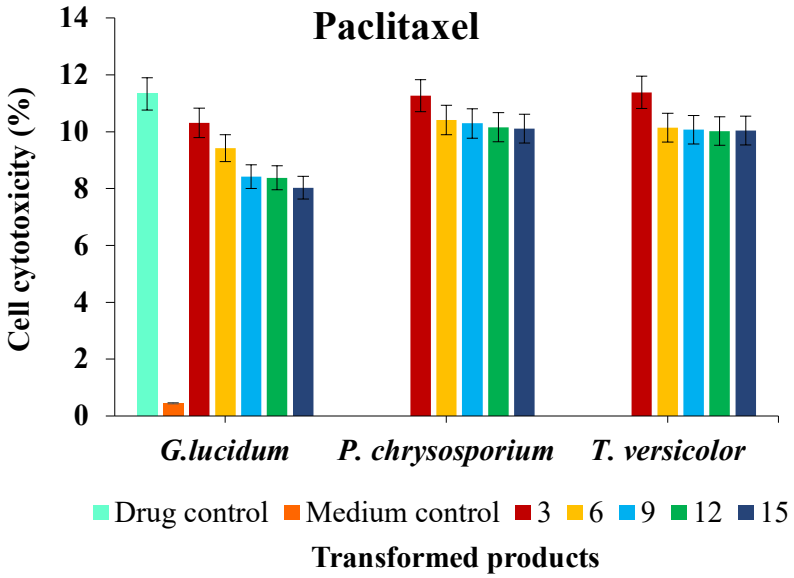


Fig. 4.29. *In-vitro* cytotoxicity of TPs of paclitaxel on Raw 264.7 cells during biodegradation with *G. lucidum*, *P. chrysosporium* and *T. versicolor*

Here, the toxicity percentage was also high on 3rd day, and it may be due to non-degradation of paclitaxel up to 3rd day. After 3 days, the toxicity level was reduced continuously at the end

of treatment experiment. When the cell cytotoxicity of native paclitaxel form was estimated, the IC_{50} value was 69.76 ±4.23 $\mu g.ml^{-1}$ between the concentration range from 10 $\mu g.ml^{-1}$ to 250 $\mu g.ml^{-1}$. If we compare to treated sample with native form, the cytotoxicity was varied but it can indicate paclitaxel is cytotoxic for Raw 264.7 cells even if present in very low concentration.

Steinbrecht et al. (2020) analysed toxicity of cyclophosphamide metabolites (4-hydroxy-cyclophosphamide) on two human cancer cell line (MCF-7 and HepG2). It was able to generate cytotoxicity on these two cell lines, but the toxicity response was more observed in MCF-7. The EC_{50} value of MCF-7 was 28 µM and HepG2 was 111 µM observed. Another study carried out by Fernandes et al. (2020) for toxicity evaluation of cyclophosphamide on *Mytilus galloprovincialis* at concentration of 1000 $ng.L^{-1}$. Cyclophosphamide was able to generate oxidative stress on murine mussels and cause DNA damage. The chronic toxicity of cyclophosphamide was evaluated on *V. fischeri* (Lutterbeck et al., 2016) as EC_{50} 1396 $\mu g.ml^{-1}$in conc. range between 1304 to 1494 $\mu g.ml^{-1}$, *Brachionus calyciflorus* as EC_{50} 89.8 $\mu g.ml^{-1}$ in conc. range between 67.2 to 119.4 $\mu g.ml^{-1}$ and *Ceriodaphnia dubia* as EC_{50} 58 $\mu g.ml^{-1}$ in conc. range between 37.4 to 89.9 $\mu g.ml^{-1}$ (Russo et al., 2018). In 2014, Parrella et al. (2014) investigated the chronic toxicity of etoposide on *B. calyciflorus, C. dubia and Daphnia magna.* The chronic toxicity of etoposide on *B. calyciflorus* measured as EC_{50} value as 3.7 $\mu g.ml^{-1}$ in conc. range between 2.7 to 5.3 $\mu g.ml^{-1}$*, C. dubia* as EC_{50} 0.204 $\mu g.ml^{-1}$ in conc. range between 0.152 to 0.256 $\mu g.ml^{-1}$ and *D. magna* as EC_{50} value 0.239 $\mu g.ml^{-1}$ in conc. range between 0.181 to 0.299 $\mu g.ml^{-1}$ observed.

These results indicate, cyclophosphamide, etoposide and paclitaxel are highly toxic for cell viability even when present in their low concentration. But after the degradation with these

fungi, the toxicity level of these compounds was reduced and their toxicity level in each fungus was varying according to their TPs generated during treatment process.

Summary and Conclusions

The present study concludes that the various antineoplastic compounds are being consumed during chemotherapy in hospitals and households by out-patients for the treatment of continuously increasing cancer cases. Antineoplastic compounds are contaminating aquatic environment and possess mutagenic, cytostatic and eco-toxicological effects on aquatic life and human health. These anti-cancer agents enter the water bodies in their original form or as metabolites via urine and faeces of the out-patients or the patients admitted in hospitals. Due to its high lipid solubility, the antineoplastic drugs accumulate in the fatty tissues of the organisms. These drugs enter through the food chain and cause health effects in humans due to their cytotoxic and genotoxic properties. Many advanced treatments such as membrane filtration, catalytic degradation etc. for the removal of these pollutants were employed but they are exhibiting certain limitations. Here, on the basis of limitations in prior said treatment technologies, the biological approach has been used for the biodegradation of some antineoplastic compounds by using white rot fungi. This approach overcome to limitation exist in physical, physio-chemical and chemical treatments.

Here, we have developed a simple, accurate, fast and reliable HPLC method for detection of the three antineoplastic compounds in environmental samples and validated the same. The developed HPLC methods have several advantages including cost effectiveness, minimal analysis time is required, and no extensive sample preparation is required. Further, use of simple solvent such as acetonitrile and water as mobile phase would not have serious environmental damage**.** Etoposide and paclitaxel were eluted best at the same developed, but they provided different retention *i.e.* 2:40.1 and 3:49.3 min. for etoposide and paclitaxel respectively. Cyclophosphamide provided best elution peak in similar solvent but retention time, mobile phase ratio and wavelength was different. The conditions of developed methods provided better resolution for all the analytes that were tested *viz* cyclophosphamide, paclitaxel and etoposide. The feasibility and reliability of methods including linearity, accuracy and

precision and robustness are good and acceptable for analysis of etoposide, paclitaxel and cyclophosphamide. Thus, we strongly propose to use the method presented here to detect cyclophosphamide, paclitaxel and etoposide in environmental water or wastewater samples.

In biodegradation study of WRFs found that, only *G. lucidum* fungus have the better potential for the removal or degradation of cyclophosphamide, etoposide and paclitaxel. Among three antineoplastic compounds treatment with white rot fungi strains, the highest biodegradation was achieved for etoposide. Etoposide was the only compounds that was removed by all three fungi. Cyclophosphamide showed degradation with only *G. lucidum* and highest biosorption efficiency with *P. chrysosporium*. The removal of paclitaxel was analysed very less in *G. lucidum* and almost negligible in *P. chrysosporium* and *T. versicolor*. This study find that the degradation carried out for these compounds in *G. lucidum* and *T. versicolor* was directly proportional to the biomass and extracellular enzyme production, while in *P. chrysosporium* it was different. The degradation of these compounds followed the pseudo first-order-kinetics study with these fungi. The maximum rate constant was calculated in degradation of etoposide with *G. lucidum*. The change in pH value and glucose utilization for each fungal culture during biodegradation were estimated. So, every WRFs does not have potential for the removal of all antineoplastic compounds and their degradation does not depend only on the biomass and enzyme activity presence, while some other factors may responsible also for the biodegradation and degradation rate.

Moreover, the study focused on toxic effect analysis of cyclophosphamide, etoposide, and paclitaxel and their TPs generated during degradation with WRFs on Raw 264.7 cell line (monocyte macrophage). MTT assay was performed to evaluate the *in-vitro* cytotoxicity and the result indicates that these compounds put an adverse effect on mouse macrophage cell line. According to the investigation all three drugs exhibiting cytotoxic effect on Raw 264.7 cells but etoposide will be classified as more toxic than paclitaxel and cyclophosphamide if present

in their native form. It indicates towards a strong effort should be made to remove or degrade these compounds at source before reaching to the aquatic environment as they are harmful for the immune system of aquatic animal diversity. While, after the degradation with selected white rot fungi, the toxicity level of these compounds reduced in the form of TPs but their toxicity was not completely eliminated still after the degradation. Further studies such as mutagenic, carcinogenic effects are required to help more understanding of the exposure and hazardous effect of these compounds on cell lines and aquatic organisms.

Following are the major conclusions in this study:

- The isocratic mode of HPLC provides the best elution for cyclophosphamide, etoposide and paclitaxel.
- The developed condition for detection and quantification of etoposide and paclitaxel were same but their retention time and peak areas were different.
- The retention time of cyclophosphamide, etoposide and paclitaxel was 3:32.3, 2:40.1, 3:49.3 min. respectively.
- The detection limit of the selected compounds in developed methods was 10 $\mu g.ml^{-1}$.
- *Ganoderma lucidum* showed the highest degradation for etoposide (>99 %), while *P. chrysosporium* showed highest biosorption efficiency for cyclophosphamide (>23 %).
- Maximum degradation rate was achieved for etoposide (k = 0.54 day^{-1}).
- *P. chrysosporium* does not showed biodegradation for cyclophosphamide, while *T. versicolor* showed only 1 % of the initial concentration of cyclophosphamide.
- The co-relation of glucose utilization and rate of degradation was not confirmed due to complete utilization of glucose during initial days of cultivation.

- The initial set pH value was changed in each case of fungal culture during the degradation of antineoplastic compounds.
- Each antineoplastic having potential to cause cytotoxic effect on Raw 264.7 cells but their toxicity limit varying.
- After the biodegradation with white rot fungi, the cytotoxicity was reduced.
- The cytotoxicity of by-products or degraded products of etoposide in treatment with *P. chrysosporium* was higher to the toxicity of by-products of etoposide with *G. lucidum* and *T. versicolor*.

Future scope of the work

In case of WRF, it should require to optimize the condition for each compounds individually with enzyme for the achievement of better degradation efficiency. Present study indicated that, the rate of degradation is not depending on the amount of biomass and enzyme activity of growing culture. So, the separate optimization should be done for the degradation of each compound by each enzyme to identify the individual effect of extracellular and intracellular enzymes on rate of degradation. In concern of toxicity, WRFs cannot eliminate the complete toxic effect of these compounds from the source even after the efficient degradation, so instead of degradation by fungi, their complete removal from water bodies should be done with no generation of toxic by-product.

Bibliography

Agrawal, N., Verma, P., Singh, R. S., and Shahi, S. K. (2017). Ganoderma lucidum: a potent source of ligninolytic enzyme production. *International Journal of Advance Research*, *5*(5), 1977-1981.

Aherne, G. W., Hardcastle, A., and Nield, A. H. (1990). Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples. *Journal of Pharmacy and Pharmacology*, *42*(10), 741-742.

Aldossary, S. A. (2019). Review on pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin. *Biomedical and Pharmacology Journal*, *12*(1), 7-15.

Allwood, M., Stanley, A., and Wright, P. (2002). *The cytotoxics handbook*: Radcliffe Publishing.

Arora, D. S., and Gill, P. K. (2001). Comparison of two assay procedures for lignin peroxidase. *Enzyme and Microbial Technology*, *28*(7-8), 602-605.

Asif, M. B., Hai, F. I., Singh, L., Price, W. E., and Nghiem, L. D. (2017). Degradation of pharmaceuticals and personal care products by white-rot fungi—A critical review. *Current Pollution Reports*, *3*(2), 88-103.

Auriol, M., Filali-Meknassi, Y., Tyagi, R. D., and Adams, C. D. (2007). Laccase-catalyzed conversion of natural and synthetic hormones from a municipal wastewater. *Water Research*, *41*(15), 3281-3288.

Azuma, T. (2018). Distribution of Anticancer Drugs in River Waters and Sediments of the Yodo River Basin, Japan. *Applied Sciences*, *8*(11), 2043.

Azuma, T., Arima, N., Tsukada, A., Hirami, S., Matsuoka, R., Moriwake, R., Ishiuchi H., Inoyama, T., Teranishi, Y., Yamaoka, M., Mino, Y., Hayashi, T., Fujita, Y., and Yamaoka, M. (2016). Detection of pharmaceuticals and phytochemicals together with

their metabolites in hospital effluents in Japan, and their contribution to sewage treatment plant influents. *Science of The Total Environment, 548*, 189-197.

Backhaus, T., Altenburger, R., Boedeker, W., Faust, M., Scholze, M., and Grime, L. H. (2000). Predictability of the toxicity of a multiple mixture of dissimilarly acting chemicals to *Vibrio fischeri*. *Environmental Toxicology and Chemistry: An International Journal, 19*(9), 2348-2356.

Bai, X., Lutz, A., Carroll, R., Keteles, K., Dahlin, K., Murphy, M., and Nguyen, D. (2018). Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds. *Chemosphere, 200*, 133-142.

Bankole, P. O., Adekunle, A. A., Jeon, B.-H., and Govindwar, S. P. (2020). Novel cobiomass degradation of NSAIDs by two wood rot fungi, *Ganoderma applanatum* and *Laetiporus sulphureus*: ligninolytic enzymes induction, isotherm and kinetic studies. *Ecotoxicology and Environmental Safety, 203*, 110997.

Bantle, J. A., Rayburn, J. R., Burton, D. T., Turley, S. D., Dawson, D. A., Dumont, J. N., Finch, R. A., Maurice, M. A., Fort, D. J., and Linder, G. (1994). Initial interlaboratory validation study of FETAX: phase I testing. *Journal of Applied Toxicology, 14*(3), 213-223.

Barisci, S., Turkay, O., Ulusoy, E., Şeker, M. G., Yüksel, E., and Dimoglo, A. (2018). Electro-oxidation of cytostatic drugs: Experimental and theoretical identification of by-products and evaluation of ecotoxicological effects. *Chemical Engineering Journal, 334*, 1820-1827.

Batt, A. L., Furlong, E. T., Mash, H. E., Glassmeyer, S. T., and Kolpin, D. W. (2017). The importance of quality control in validating concentrations of contaminants of emerging concern in source and treated drinking water samples. *Science of The Total Environment, 579*, 1618-1628.

Besse, J.-P., Latour, J.-F., and Garric, J. (2012). Anticancer drugs in surface waters: what can we say about the occurrence and environmental significance of cytotoxic, cytostatic and endocrine therapy drugs? *Environment International, 39*(1), 73-86.

Białk-Bielińska, A., Mulkiewicz, E., Stokowski, M., Stolte, S., and Stepnowski, P. (2017). Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery–biological effects and stability under test conditions. *Chemosphere, 189*, 689-698.

Bhatt, P., Zhang, W., Lin, Z., Pang, S., Huang, Y., and Chen, S. (2020). Biodegradation of allethrin by a novel fungus *Fusarium proliferatum* strain CF_2, isolated from contaminated soils. *Microorganisms*, *8*(4), 593.

Buchan, B. W., Relich, R. F., and Mahlen, S. D. (2019). Interim Clinical Laboratory Guideline for Biological Safety. *The Amercian Society for Microbiology*. (Source: https://asm.org/Articles/2019/January/Interim-Clinical-Laboratory-Guideline-for-Biologic).

Booker, V., Halsall, C., Llewellyn, N., Johnson, A., and Williams, R. (2014). Prioritising anticancer drugs for environmental monitoring and risk assessment purposes. *Science of The Total Environment, 473*, 159-170.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394-424.

Broséus, R., Vincent, S., Aboulfadl, K., Daneshvar, A., Sauvé, S., Barbeau, B., and Prévost, M. (2009). Ozone oxidation of pharmaceuticals, endocrine disruptors and pesticides during drinking water treatment. *Water Research, 43*(18), 4707-4717.

Buerge, I. J., Buser, H.-R., Poiger, T., and Müller, M. D. (2006). Occurrence and fate of the cytostatic drugs cyclophosphamide and ifosfamide in wastewater and surface waters. *Environmental Science and Technology, 40*(23), 7242-7250.

Calafat, A., and Sampson, E. (2012). Laboratory procedure manual: bisphenol A and other environmental phenols and parabens in urine. Method number 6301.01. (Source: cdc.gov/nchs).

Calza, P., Medana, C., Sarro, M., Rosato, V., Aigotti, R., Baiocchi, C., and Minero, C. (2014). Photocatalytic degradation of selected anticancer drugs and identification of their transformation products in water by liquid chromatography–high resolution mass spectrometry. *Journal of Chromatography A, 1362*, 135-144.

Caminada, D., Zaja, R., Smital, T., and Fent, K. (2008). Human pharmaceuticals modulate P-gp1 (ABCB1) transport activity in the fish cell line PLHC-1. *Aquatic Toxicology, 90*(3), 214-222.

Castegnaro, M., De Méo, M., Laget, M., Michelon, J., Garren, L., Sportouch, M., and Hansel, S. (1997). Chemical degradation of wastes of antineoplastic agents. *International Archives of Occupational and Environmental Health*, *70*(6), 378-384.

Castellet-Rovira, F., Lucas, D., Villagrasa, M., Rodríguez-Mozaz, S., Barceló, D., and Sarrà, M. (2018). *Stropharia rugosoannulata* and *Gymnopilus luteofolius*: Promising fungal species for pharmaceutical biodegradation in contaminated water. *Journal of Environmental Management*, *207*, 396-404.

Catastini, C., Mullot, J.-U., Boukari, S., Mazellier, P., Levi, Y., Cervantes, P., and Ormsby, J.N. (2008). Identification de molécules anticancéreuses dans les effluents hospitaliers. *European Journal of Water Quality, 39*(2), 171-180.

CDER, F. (1996). Guidance for industry: single dose acute toxicity testing for pharmaceuticals (Final). (Source: http://www. fda. gov, 1996).

Česen, M., Eleršek, T., Novak, M., Žegura, B., Kosjek, T., Filipič, M., and Heath, E. (2016). Ecotoxicity and genotoxicity of cyclophosphamide, ifosfamide, their metabolites/transformation products and their mixtures. *Environmental Pollution, 210*, 192-201.

Česen, M., Kosjek, T., Busetti, F., Kompare, B., and Heath, E. (2016). Human metabolites and transformation products of cyclophosphamide and ifosfamide: analysis, occurrence and formation during abiotic treatments. *Environmental Science and Pollution Research, 23*(11), 11209-11223.

Česen, M., Kosjek, T., Laimou-Geraniou, M., Kompare, B., Širok, B., Lambropolou, D., and Heath, E. (2015). Occurrence of cyclophosphamide and ifosfamide in aqueous environment and their removal by biological and abiotic wastewater treatment processes. *Science of The Total Environment, 527*, 465-473.

Chen, S., Blaney, L., Chen, P., Deng, S., Hopanna, M., Bao, Y., and Yu, G. (2019). Ozonation of the 5-fluorouracil anticancer drug and its prodrug capecitabine: Reaction kinetics, oxidation mechanisms, and residual toxicity. *Frontiers of Environmental Science and Engineering*, *13*(4), 59.

Coetsier, C., Spinelli, S., Lin, L., Roig, B., and Touraud, E. (2009). Discharge of pharmaceutical products (PPs) through a conventional biological sewage treatment plant: MECs vs PECs? *Environment International, 35*(5), 787-792.

Couto, S. R., Moldes, D., and Sanroman, M. A. (2006). Optimum stability conditions of pH and temperature for ligninase and manganese-dependent peroxidase from *Phanerochaete chrysosporium*. Application to in vitro decolorization of Poly R-478 by MnP. *World Journal of Microbiology and Biotechnology*, *22*(6), 607-612.

Cristóvão, M., Janssens, R., Yadav, A., Pandey, S., Luis, P., Van der Bruggen, B., Dubey, K.K., Mandal, M.K., Crespo, J.G., and Pereira, V. (2020). Predicted concentrations of

anticancer drugs in the aquatic environment: What should we monitor and where should we treat? *Journal of Hazardous Materials*, 122330.

Cristóvão, M., Torrejais, J., Janssens, R., Luis, P., Van der Bruggen, B., Dubey, K.K, Mandal, M.K., Bronze, M.R., Crespo, J.G., and Pereira, V. (2019). Treatment of anticancer drugs in hospital and wastewater effluents using nanofiltration. *Separation and Purification Technology, 224*, 273-280.

Cruz-Morató, C., Lucas, D., Llorca, M., Rodriguez-Mozaz, S., Gorga, M., Petrovic, M., Barcelo, D., Vicent, T., Sarra, M., and Marco-Urrea, E. (2014). Hospital wastewater treatment by fungal bioreactor: removal efficiency for pharmaceuticals and endocrine disruptor compounds. *Science of The Total Environment, 493*, 365-376.

da Costa Araújo, A. P., Mesak, C., Montalvão, M. F., Freitas, Í. N., Chagas, T. Q., and Malafaia, G. (2019). Anti-cancer drugs in aquatic environment can cause cancer: insight about mutagenicity in tadpoles. *Science of The Total Environment, 650*, 2284-2293.

da Rosa, A. P. P., Cavalcante, R. P., da Silva, D. A., da Silva, L. d. M., da Silva, T. F., Gozzi, F., McGlynn, E., Brady-Boyd, A., Casagrande, G.A., Wender, H., Cesar-de-Oliveira, S., and Junior, A.M. (2019). H2O2-assisted photoelectrocatalytic degradation of Mitoxantrone using CuO nanostructured films: Identification of by-products and toxicity. *Science of The Total Environment, 651*, 2845-2856.

Das, M. T., Budhraja, V., Mishra, M., and Thakur, I. S. (2012). Toxicological evaluation of paper mill sewage sediment treated by indigenous dibenzofuran-degrading Pseudomonas sp. *Bioresource Technology, 110*, 71-78.

Dehghanpour, S., Pourzamani, H. R., Amin, M. M., and Ebrahimpour, K. (2020). Evaluation of toxic effects of platinum-based antineoplastic drugs (Cisplatin, Carboplatin and Oxaliplatin) on green alga Chlorella vulgaris. *Aquatic Toxicology*, 105495.

Delgado, L. F., Faucet-Marquis, V., Pfohl-Leszkowicz, A., Dorandeu, C., Marion, B., Schetrite, S., and Albasi, C. (2011). Cytotoxicity micropollutant removal in a crossflow membrane bioreactor. *Bioresource Technology, 102*(6), 4395-4401.

DeYoung, D., Bantle, J., Hull, M., and Burks, S. (1996). Differences in sensitivity to developmental toxicants as seen in *Xenopus* and *Pimephales* embryos. *Bulletin of Environmental Contamination and Toxicology, 56*(1), 143-150.

Du, B., Haddad, S. P., Luek, A., Scott, W. C., Saari, G. N., Kristofco, L. A., Connors, K. A., Rash, C., Rasmussen, J. B., Chambliss, C. K., and Brooks, B. W. (2014). Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*(1656), 20140058.

Dubey, K. K., Kumar, P., Labrou, N. E., and Shukla, P. (2017). Biotherapeutic potential and mechanisms of action of colchicine. *Critical Reviews in Biotechnology, 37*(8), 1038-1047.

Eibes, G., Debernardi, G., Feijoo, G., Moreira, M. T., and Lema, J. M. (2011). Oxidation of pharmaceutically active compounds by a ligninolytic fungal peroxidase. *Biodegradation, 22*(3), 539-550.

El-Kady, A. A., and Abdel-Wahhab, M. A. (2018). Occurrence of trace metals in foodstuffs and their health impact. *Trends in Food Science and Technology, 75*, 36-45.

Elersek, T., Milavec, S., Korošec, M., Brezovsek, P., Negreira, N., Zonja, B., Lopez-de-Alda, M., Barcelo, D., Ester, H., Ščančar, J. and Filipic, M. (2016). Toxicity of the mixture of selected antineoplastic drugs against aquatic primary producers. *Environmental Science and Pollution Research, 23*(15), 14780-14790.

Fabiańska, A., Ofiarska, A., Fiszka-Borzyszkowska, A., Stepnowski, P., and Siedlecka, E. M. (2015). Electrodegradation of ifosfamide and cyclophosphamide at BDD electrode: Decomposition pathway and its kinetics. *Chemical Engineering Journal, 276*, 274-282.

Ferrando-Climent, L., Cruz-Morató, C., Marco-Urrea, E., Vicent, T., Sarrà, M., Rodriguez-Mozaz, S., and Barceló, D. (2015). Non conventional biological treatment based on *Trametes versicolor* for the elimination of recalcitrant anticancer drugs in hospital wastewater. *Chemosphere, 136*, 9-19.

Ferrando-Climent, L., Gonzalez-Olmos, R., Anfruns, A., Aymerich, I., Corominas, L., Barceló, D., and Rodriguez-Mozaz, S. (2017). Elimination study of the chemotherapy drug tamoxifen by different advanced oxidation processes: Transformation products and toxicity assessment. *Chemosphere, 168*, 284-292.

Ferrando-Climent, L., Rodriguez-Mozaz, S., and Barceló, D. (2013). Development of a UPLC-MS/MS method for the determination of ten anticancer drugs in hospital and urban wastewaters, and its application for the screening of human metabolites assisted by information-dependent acquisition tool (IDA) in sewage samples. *Analytical and bioanalytical chemistry, 405*(18), 5937-5952.

Ferrando-Climent, L., Rodriguez-Mozaz, S., and Barceló, D. (2014). Incidence of anticancer drugs in an aquatic urban system: From hospital effluents through urban wastewater to natural environment. *Environmental Pollution, 193*, 216-223.

Ferrando Climent, L. (2016). Analysis of chemotherapy drugs and related compounds in aquatic environment: removal, transformation and risk evaluation in eco-friendly and advanced technologies. (Source: https://dugi-doc.udg.edu)

Ferre-Aracil, J., Valcárcel, Y., Negreira, N., de Alda, M. L., Barceló, D., Cardona, S., and Navarro-Laboulais, J. (2016). Ozonation of hospital raw wastewaters for cytostatic

compounds removal. Kinetic modelling and economic assessment of the process. *Science of The Total Environment, 556*, 70-79.

Filipič, M. (2014). Fate and Effects of Cytostatic Pharmaceuticals in the Environment and the Identification of Biomarkers for and Improved Risk Assessment on Environmental Exposure: European commission, Research and innovation. (Source: https://cordis.europa.eu/project/id/265264/results)

Franquet-Griell, H., Medina, A., Sans, C., and Lacorte, S. (2017). Biological and photochemical degradation of cytostatic drugs under laboratory conditions. *Journal of Hazardous Materials, 323*, 319-328.

Garcia-Ac, A., Broséus, R., Vincent, S., Barbeau, B., Prévost, M., and Sauvé, S. (2010). Oxidation kinetics of cyclophosphamide and methotrexate by ozone in drinking water. *Chemosphere, 79*(11), 1056-1063.

García, L. F., Lozano, O. L., Lorenzo, E. V., Venta, M. B., Rodríguez, Y. R., Castro, C. H., Trujillo, C. G., and Torres, I. F. (2020). Review of cytostatic wastewater degradation by ozone and advanced oxidation processes: results from Cuban studies. *Environmental Reviews, 28*(1), 21-31.

Garriga, M., Almaraz, M., and Marchiaro, A. (2017). Determination of reducing sugars in extracts of Undaria pinnatifida (harvey) algae by UV-visible spectrophotometry (DNS method). *Desarro. E Innovación En Ing*, 444-454.

Gerlak, A. K., House-Peters, L., Varady, R. G., Albrecht, T., Zúñiga-Terán, A., de Grenade, R. R., Cook, C., and Scott, C. A. (2018). Water security: A review of place-based research. *Environmental Science and Policy, 82*, 79-89.

Ghafuri, Y., Yunesian, M., Nabizadeh, R., Mesdaghinia, A., Dehghani, M., and Alimohammadi, M. (2018). Platinum cytotoxic drugs in the municipal wastewater and

drinking water, a validation method and health risk assessment. *Human and Ecological Risk Assessment: An International Journal, 24*(3), 784-796.

Ghafuria, Y., Yunesian, M., Nabizadeh, R., Mesdaghinia, A., Dehghani, M., and Alimohammadi, M. (2018). Environmental risk assessment of platinum cytotoxic drugs: a focus on toxicity characterization of hospital effluents. *International Journal of Environmental Science and Technology, 15*(9), 1983-1990.

Golan-Rozen, N., Chefetz, B., Ben-Ari, J., Geva, J., and Hadar, Y. (2011). Transformation of the recalcitrant pharmaceutical compound carbamazepine by *Pleurotus ostreatus*: role of cytochrome P450 monooxygenase and manganese peroxidase. *Environmental Science and Technology, 45*(16), 6800-6805.

Gómez-Canela, C., Cortés-Francisco, N., Oliva, X., Pujol, C., Ventura, F., Lacorte, S., and Caixach, J. (2012). Occurrence of cyclophosphamide and epirubicin in wastewaters by direct injection analysis–liquid chromatography–high-resolution mass spectrometry. *Environmental Science and Pollution Research, 19*(8), 3210-3218.

Gómez-Canela, C., Ventura, F., Caixach, J., and Lacorte, S. (2014). Occurrence of cytostatic compounds in hospital effluents and wastewaters, determined by liquid chromatography coupled to high-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry, 406*(16), 3801-3814.

Goullé, J.-P., Saussereau, E., Mahieu, L., Cellier, D., Spiroux, J., and Guerbet, M. (2012). Importance of anthropogenic metals in hospital and urban wastewater: its significance for the environment. *Bulletin of Environmental Contamination and Toxicology, 89*(6), 1220-1224.

Gouveia, T. I., Alves, A., and Santos, M. S. (2019). New insights on cytostatic drug risk assessment in aquatic environments based on measured concentrations in surface waters. *Environment International, 133*, 105236.

Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., and Doumenq, P. (2017). From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review. *Water Research, 111*, 297-317.

Grelska, A., and Noszczyńska, M. (2020). White rot fungi can be a promising tool for removal of bisphenol A, bisphenol S, and nonylphenol from wastewater. *Environmental Science and Pollution Research*, 1-19.

Grung, M., Källqvist, T., Sakshaug, S., Skurtveit, S., and Thomas, K. V. (2008). Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. *Ecotoxicology and Environmental Safety, 71*(2), 328-340.

Gu, G., Yin, H., Zhu, Q., Shen, L., Zhang, K., Liu, M., and Wu, Q. (2019). Recognition of the prioritized types and individual of pharmaceuticals and personal care products (PPCPs) in the drinking water of Shanghai and a health risk assessment. *Human and Ecological Risk Assessment: An International Journal, 25*(5), 1207-1221.

Guo, R., Zheng, F., and Chen, J. (2015). Evaluation of the aquatic toxic effect varied during the degradation of capecitabine under the environmental abiotic and biotic processes. *RSC Advances, 5*(94), 76772-76778.

Guzel, E. Y., Cevik, F., and Daglioglu, N. (2018). Determination of pharmaceutical active compounds in Ceyhan River, Turkey: Seasonal, spatial variations and environmental risk assessment. *Human and Ecological Risk Assessment: An International Journal*, 1-16.

Habibzadeh, M., Chaibakhsh, N., and Naeemi, A. S. (2018). Optimized treatment of wastewater containing cytotoxic drugs by living and dead biomass of the freshwater microalga, *Chlorella vulgaris*. *Ecological Engineering, 111*, 85-93.

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P. F., Ingerslev, F., Holten Lützhøft, H. C., and Jørgensen, S. E. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment- A review. *Chemosphere, 36*(2), 357-393.

Hann, S., Stefánka, Z., Lenz, K., and Stingeder, G. (2005). Novel separation method for highly sensitive speciation of cancerostatic platinum compounds by HPLC–ICP–MS. *Analytical and Bioanalytical Chemistry, 381*(2), 405-412.

Hansel, S., Castegnaro, M., Sportouch, M., De Meo, M., Milhavet, J., Laget, M., and Dumenil, G. (1996). Chemical degradation of wastes of antineoplastic agents: cyclophosphamide, ifosfamide and melphalan. *International Archives of Occupational and Environmental Health, 69*(2), 109-114.

Hariharan, H., and Padma, N. (2013). Optimization of lignin peroxidase, manganese peroxidase, and lac production from *Ganoderma lucidum* under solid state fermentation of pineapple leaf. *Bioresources*, 8(1), 250-271.

Haroune, L., Saibi, S., Bellenger, J.-P., and Cabana, H. (2014). Evaluation of the efficiency of Trametes hirsuta for the removal of multiple pharmaceutical compounds under low concentrations relevant to the environment. *Bioresource Technology, 171*, 199-202.

Heath, E., Isidori, M., Kosjek, T., and Filipič, M. (2020). Fate and effects of anticancer drugs in the environment: Springer Nature, 469.

Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters, 131*(1-2), 5-17.

Henschel, K.-P., Wenzel, A., Diedrich, M., and Fliedner, A. (1997). Environmental hazard assessment of pharmaceuticals. *Regulatory toxicology and Pharmacology, 25*(3), 220-225.

Hernando, M. D., Mezcua, M., Fernández-Alba, A. R., and Barceló, D. (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta, 69*(2), 334-342.

Heynen, N. (2016). Urban political ecology II: The abolitionist century. *Progress in Human Geography, 40*(6), 839-845.

Hirose, J., Kondo, F., Nakano, T., Kobayashi, T., Hiro, N., Ando, Y., Takenaka, H., and Sano, K. (2005). Inactivation of antineoplastics in clinical wastewater by electrolysis. *Chemosphere, 60*(8), 1018-1024.

Huo, Z., Wang, S., Shao, H., Wang, H., and Xu, G. (2020). Radiolytic degradation of anticancer drug capecitabine in aqueous solution: kinetics, reaction mechanism, and toxicity evaluation. *Environmental Science and Pollution Research*, 1-10.

Isidori, M., Lavorgna, M., Russo, C., Kundi, M., Žegura, B., Novak, M., Filipic, M., Misik, M., Knasmueller, S., de Alda, M. L., Barcelo, D., Zonja, B., Cesen, M., Scancar, J., Kosjek, T., and Heath, E. (2016). Chemical and toxicological characterisation of anticancer drugs in hospital and municipal wastewaters from Slovenia and Spain. *Environmental pollution, 219*, 275-287.

Janssens, R., Cristóvão, B. M., Bronze, M. R., Crespo, J. G., Pereira, V. J., and Luis, P. (2020). Photocatalysis Using UV-A and UV-C Light Sources for Advanced Oxidation of Anti-Cancer Drugs Spiked in Laboratory-Grade Water and Synthetic Urine. *Industrial and Engineering Chemistry Research, 59*(2), 647-653.

Janssens, R., Cristovao, M., Bronze, M., Crespo, J., Pereira, V., and Luis, P. (2019). Coupling of nanofiltration and UV, UV/TiO_2 and UV/H_2O_2 processes for the removal of anti-cancer drugs from real secondary wastewater effluent. *Journal of Environmental Chemical Engineering, 7*(5), 103351.

Janssens, R., Mandal, M. K., Dubey, K. K., and Luis, P. (2017). Slurry photocatalytic membrane reactor technology for removal of pharmaceutical compounds from wastewater: Towards cytostatic drug elimination. *Science of The Total Environment, 599*, 612-626.

Jayasinghe, C., Imtiaj, A., Hur, H., Lee, G. W., Lee, T. S., & Lee, U. Y. (2008). Favourable culture conditions for mycelial growth of Korean wild strains in *Ganoderma lucidum*. *Mycobiology*, *36*(1), 28-33.

Jensen, K. M., Kahl, M. D., Makynen, E. A., Korte, J. J., Leino, R. L., Butterworth, B. C., and Ankley, G. T. (2004). Characterization of responses to the antiandrogen flutamide in a short-term reproduction assay with the fathead minnow. *Aquatic Toxicology, 70*(2), 99-110.

Johnson, A. C., Jürgens, M. D., Williams, R. J., Kümmerer, K., Kortenkamp, A., and Sumpter, J. P. (2008). Do cytotoxic chemotherapy drugs discharged into rivers pose a risk to the environment and human health? An overview and UK case study. *Journal of Hydrology, 348*(1-2), 167-175.

Jones, B., Little, A. C., Boothroyd, L., Debruine, L. M., Feinberg, D. R., Smith, M. J., Cornwell, R. E., Moore, F. R., and Perrett, D. I. (2005). Commitment to relationships and preferences for femininity and apparent health in faces are strongest on days of the menstrual cycle when progesterone level is high. *Hormones and Behaviour, 48*(3), 283-290.

Junnarkar, N., Pandhi, N., Raiyani, N., Bhatt, N., and Raiyani, R. (2016). Production of LiP by *Phanerochaete chrysosporium* MTCC-787 through solid state fermentation of wheat straw and assessing its activity against reactive black B. *International Journal of Advance Research,* 4(1), 812-819.

Jureczko, M., and Kalka, J. (2020). Cytostatic pharmaceuticals as water contaminants. *European Journal of Pharmacology, 866*, 172816.

Jureczko, M., and Przystaś, W. (2019). Ecotoxicity risk of presence of two cytostatic drugs: bleomycin and vincristine and their binary mixture in aquatic environment. *Ecotoxicology and Environmental Safety, 172*, 210-215.

Jureczko, M., Przystaś, W., Krawczyk, T., Gonciarz, W., and Rudnicka, K. (2021). White-rot fungi-mediated biodegradation of cytostatic drugs-bleomycin and vincristine. *Journal of Hazardous Materials, 407*, 124632.

Kelbert, M., Pereira, C. S., Daronch, N. A., Cesca, K., Michels, C., de Oliveira, D., and Soares, H. M. (2021). Laccase as an efficacious approach to remove anticancer drugs: A study of doxorubicin degradation, kinetic parameters, and toxicity assessment. *Journal of Hazardous Materials, 409*, 124520.

Kim, I., Yamashita, N., and Tanaka, H. (2009). Photodegradation of pharmaceuticals and personal care products during UV and UV/H_2O_2 treatments. *Chemosphere, 77*(4), 518-525.

Kirk, T. K., Schultz, E., Connors, W., Lorenz, L., and Zeikus, J. (1978). Influence of culture parameters on lignin metabolism by Phanerochaete chrysosporium. *Archives of Microbiology, 117*(3), 277-285.

Kobayashi, T., Hirose, J., Sano, K., Kato, R., Ijiri, Y., Takiuchi, H., Tanaka, H., Goto, E., Tamai, H., and Nakano, T. (2012). Application of electrolysis for detoxification of an antineoplastic in urine. *Ecotoxicology and Environmental Safety, 78*, 123-127.

Koltsakidou, A., Antonopoulou, M., Evgenidou, E., Konstantinou, I., and Lambropoulou, D. (2017). Cytarabine degradation by simulated solar assisted photocatalysis using TiO_2. *Chemical Engineering Journal, 316*, 823-831.

Kosjek, T., and Heath, E. (2011). Occurrence, fate and determination of cytostatic pharmaceuticals in the environment. *TrAC Trends in Analytical Chemistry, 30*(7), 1065-1087.

Kosjek, T., Negreira, N., Heath, E., de Alda, M. L., and Barceló, D. (2018). Aerobic activated sludge transformation of vincristine and identification of the transformation products. *Science of The Total Environment, 610*, 892-904.

Kosjek, T., Perko, S., Žigon, D., and Heath, E. (2013). Fluorouracil in the environment: analysis, occurrence, degradation and transformation. *Journal of Chromatography A, 1290*, 62-72.

Kovács, R., Bakos, K., Urbányi, B., Kövesi, J., Gazsi, G., Csepeli, A., Appl, A. J., Bencsik, D., Csenki, Z., and Horváth, Á. (2016). Acute and sub-chronic toxicity of four cytostatic drugs in zebrafish. *Environmental Science and Pollution Research, 23*(15), 14718-14729.

Kovalova, L., McArdell, C. S., and Hollender, J. (2009). Challenge of high polarity and low concentrations in analysis of cytostatics and metabolites in wastewater by hydrophilic interaction chromatography/tandem mass spectrometry. *Journal of Chromatography A, 1216*(7), 1100-1108.

Kovalova, L., Siegrist, H., Von Gunten, U., Eugster, J., Hagenbuch, M., Wittmer, A., Moser, R., and McArdell, C. S. (2013). Elimination of micropollutants during post-treatment of hospital wastewater with powdered activated carbon, ozone, and UV. *Environmental Science and Technology, 47*(14), 7899-7908.

Kumar, P. (2019). Numerical quantification of current status quo and future prediction of water quality in eight Asian megacities: challenges and opportunities for sustainable water management. *Environmental Monitoring and Assessment, 191*(6), 1-12.

Kümmerer, K. (2001). Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources–a review. *Chemosphere, 45*(6-7), 957-969.

Kümmerer, K., Haiß, A., Schuster, A., Hein, A., and Ebert, I. (2016). Antineoplastic compounds in the environment—substances of special concern. *Environmental Science and Pollution Research, 23*(15), 14791-14804.

Kümmerer, K., Steger-Hartmann, T., and Meyer, M. (1997). Biodegradability of the anti-tumour agent ifosfamide and its occurrence in hospital effluents and communal sewage. *Water Research, 31*(11), 2705-2710.

Kuwahara, M., Glenn, J. K., Morgan, M. A., and Gold, M. H. (1984). Separation and characterization of two extracelluar H_2O_2-dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium*. *FEBS letters, 169*(2), 247-250.

Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P., and Klaminder, J. (2016). Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web-Insights from a field experiment. *Science of The Total Environment, 568*, 208-215.

Lai, W. W.-P., Lin, H. H.-H., and Lin, A. Y.-C. (2015). TiO_2 photocatalytic degradation and transformation of oxazaphosphorine drugs in an aqueous environment. *Journal of Hazardous Materials, 287*, 133-141.

Larson, R. R., Khazaeli, M., and Dillon, H. K. (2003). Development of an HPLC method for simultaneous analysis of five antineoplastic agents. *Applied Occupational and Environmental Hygiene, 18*(2), 109-119.

Lazarova, Z., and Spendlingwimmer, R. (2008). Treatment of yellow water by membrane separations and advanced oxidation methods. *Water Science and Technology, 58*(2), 419-426.

Lenz, K., Mahnik, S., Weissenbacher, N., Mader, R., Krenn, P., Hann, S., Koellensperger, G., Uhl, S., Knasmuller, S., Ferk, F., and Fuerhacker, M. (2007). Monitoring, removal and risk assessment of cytostatic drugs in hospital wastewater. *Water Science and Technology, 56*(12), 141-149.

Lester, Y., Avisar, D., Gozlan, I., and Mamane, H. (2011). Removal of pharmaceuticals using combination of UV/H_2O_2/O_2 advanced oxidation process. *Water Science and Technology, 64*(11), 2230-2238.

Li, W., Nanaboina, V., Chen, F., and Korshin, G. V. (2016). Removal of polycyclic synthetic musks and antineoplastic drugs in ozonated wastewater: quantitation based on the data of differential spectroscopy. *Journal of Hazardous Materials, 304*, 242-250.

Lin, A. Y.-C., Hsueh, J. H.-F., and Hong, P. A. (2015). Removal of antineoplastic drugs cyclophosphamide, ifosfamide, and 5-fluorouracil and a vasodilator drug pentoxifylline from wastewaters by ozonation. *Environmental Science and Pollution Research, 22*(1), 508-515.

Lin, H. H.-H., and Lin, A. Y.-C. (2014). Photocatalytic oxidation of 5-fluorouracil and cyclophosphamide via UV/TiO_2 in an aqueous environment. *Water Research, 48*, 559-568.

Liu, X., Zhang, J., Yin, J., Duan, H., Wu, Y., and Shao, B. (2010). Analysis of hormone antagonists in clinical and municipal wastewater by isotopic dilution liquid chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 396*(8), 2977-2985.

Lojk, J., Repas, J., Veranič, P., Bregar, V. B., and Pavlin, M. (2020). Toxicity mechanisms of selected engineered nanoparticles on human neural cells in vitro. *Toxicology*, 152364.

Lucas, D., Castellet-Rovira, F., Villagrasa, M., Badia-Fabregat, M., Barceló, D., Vicent, T., Caminel, G., Sarra, M., and Rodríguez-Mozaz, S. (2018). The role of sorption processes

in the removal of pharmaceuticals by fungal treatment of wastewater. *Science of The Total Environment, 610*, 1147-1153.

Lutterbeck, C. A., Baginska, E., Machado, Ê. L., and Kümmerer, K. (2015). Removal of the anti-cancer drug methotrexate from water by advanced oxidation processes: aerobic biodegradation and toxicity studies after treatment. *Chemosphere, 141*, 290-296.

Lutterbeck C.A., Machado Ê.L., Kümmerer K. (2020). Degradation and elimination of anticancer drugs by water and wastewater treatment – toxicity and biodegradability before and after the treatment. In: Heath E., Isidori M., Kosjek T., Filipič M. (eds) *Fate and Effects of Anticancer Drugs in the Environment*. Springer, Cham.

Lutterbeck, C. A., Wilde, M. L., Baginska, E., Leder, C., Machado, Ê. L., and Kümmerer, K. (2015). Degradation of 5-FU by means of advanced (photo) oxidation processes: UV/H_2O_2, UV/Fe^{2+}-H_2O_2 and UV/TiO_2—comparison of transformation products, ready biodegradability and toxicity. *Science of The Total Environment, 527*, 232-245.

Lutterbeck, C. A., Wilde, M. L., Baginska, E., Leder, C., Machado, Ê. L., and Kümmerer, K. (2016). Degradation of cyclophosphamide and 5-fluorouracil by UV and simulated sunlight treatments: assessment of the enhancement of the biodegradability and toxicity. *Environmental Pollution, 208*, 467-476.

Mahnik, S., Lenz, K., Weissenbacher, N., Mader, R., and Fuerhacker, M. (2007). Fate of 5-fluorouracil, doxorubicin, epirubicin, and daunorubicin in hospital wastewater and their elimination by activated sludge and treatment in a membrane-bio-reactor system. *Chemosphere, 66*(1), 30-37.

Mahnik, S. N., Rizovski, B., Fuerhacker, M., and Mader, R. M. (2004). Determination of 5-fluorouracil in hospital effluents. *Analytical and Bioanalytical Chemistry, 380*(1), 31-35.

Mahnik, S. N., Rizovski, B., Fuerhacker, M., and Mader, R. M. (2006). Development of an analytical method for the determination of anthracyclines in hospital effluents. *Chemosphere, 65*(8), 1419-1425.

Marco-Urrea, E., Pérez-Trujillo, M., Vicent, T., and Caminal, G. (2009). Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by Trametes versicolor. *Chemosphere, 74*(6), 765-772.

Márta, Z., Bobály, B., Fekete, J., Magda, B., Imre, T., Mészáros, K. V., Balint, M., and Szabó, P. T. (2018). Simultaneous determination of thirteen different steroid hormones using micro UHPLC-MS/MS with on-line SPE system. *Journal of Pharmaceutical and Biomedical Analysis, 150*, 258-267.

Martín, J., Camacho-Muñoz, D., Santos, J. L., Aparicio, I., and Alonso, E. (2014). Occurrence and ecotoxicological risk assessment of 14 cytostatic drugs in wastewater. *Water, Air, and Soil Pollution, 225*(3), 1896.

Martín, J., Camacho-Muñoz, D., Santos, J. L., Aparicio, I., and Alonso, E. (2011). Simultaneous determination of a selected group of cytostatic drugs in water using high-performance liquid chromatography–triple-quadrupole mass spectrometry. *Journal of Separation Science, 34*(22), 3166-3177.

Mastroianni, N., Bleda, M. J., de Alda, M. L., and Barceló, D. (2016). Occurrence of drugs of abuse in surface water from four Spanish river basins: Spatial and temporal variations and environmental risk assessment. *Journal of Hazardous Materials, 316*, 134-142.

Mater, N., Geret, F., Castillo, L., Faucet-Marquis, V., Albasi, C., and Pfohl-Leszkowicz, A. (2014). In vitro tests aiding ecological risk assessment of ciprofloxacin, tamoxifen and cyclophosphamide in range of concentrations released in hospital wastewater and surface water. *Environment international, 63*, 191-200.

McKnight, J. A. (2003). Principles of chemotherapy. *Clinical techniques in small animal practice, 18*(2), 67-72.

Mir-Tutusaus, J. A., Baccar, R., Caminal, G., and Sarrà, M. (2018). Can white-rot fungi be a real wastewater treatment alternative for organic micropollutants removal? A review. *Water Research, 138*, 137-151.

Mir-Tutusaus, J. A., Sarrà, M., and Caminal, G. (2016). Continuous treatment of non-sterile hospital wastewater by *Trametes versicolor*: how to increase fungal viability by means of operational strategies and pretreatments. *Journal of Hazardous Materials, 318,* 561-570.

Mišík, M., Filipic, M., Nersesyan, A., Kundi, M., Isidori, M., and Knasmueller, S. (2019). Environmental risk assessment of widely used anticancer drugs (5-fluorouracil, cisplatin, etoposide, imatinib mesylate). *Water Research, 164*, 114953.

Montgomery, M. A., and Elimelech, M. (2007). Water and sanitation in developing countries: including health in the equation. *Environmental Science and Technology, 41*(1), 17-24.

Mukherjee, S., Mehta, D., Dhangar, K., and Kumar, M. (2020). Environmental Fate, Distribution and State-of-the-art Removal of Antineoplastic Drugs: A Comprehensive Insight. *Chemical Engineering Journal*, 127184.

Naghdi, M., Taheran, M., Brar, S. K., Kermanshahi-Pour, A., Verma, M., and Surampalli, R. Y. (2018). Removal of pharmaceutical compounds in water and wastewater using fungal oxidoreductase enzymes. *Environmental Pollution, 234*, 190-213.

Nakano, M., Nakazawa, M., Sasaki, Y., Takeuchi, E., Miho, Y., Ishikawa, H., . . . Watanabe, A. (2013). Portable information terminal, display control device, display control method, and computer readable program therefore: Google Patents. (Source: https://patents.google.com/patent/US8375334B2/en).

Nassour, C., Barton, S. J., Nabhani-Gebara, S., Saab, Y., and Barker, J. (2019). Occurrence of anticancer drugs in the aquatic environment: a systematic review. *Environmental Science and Pollution Research*, 1-9.

Nasreen, Z., Kausar, T., Nadeem, M., & Bajwa, R. (2005). Study of different growth parameters in *Ganoderma lucidum*. *Micología Aplicada International*, *17*(1), 5-8.

Negreira, N., de Alda, M. L., and Barceló, D. (2013). On-line solid phase extraction–liquid chromatography–tandem mass spectrometry for the determination of 17 cytostatics and metabolites in waste, surface and ground water samples. *Journal of Chromatography A, 1280*, 64-74.

Negreira, N., de Alda, M. L., and Barceló, D. (2014a). Cytostatic drugs and metabolites in municipal and hospital wastewaters in Spain: filtration, occurrence, and environmental risk. *Science of The Total Environment, 497*, 68-77.

Negreira, N., de Alda, M. L., and Barceló, D. (2014b). Study of the stability of 26 cytostatic drugs and metabolites in wastewater under different conditions. *Science of The Total Environment, 482*, 389-398.

Novak, M., Žegura, B., Modic, B., Heath, E., and Filipič, M. (2017). Cytotoxicity and genotoxicity of anticancer drug residues and their mixtures in experimental model with zebrafish liver cells. *Science of The Total Environment, 601*, 293-300.

Nussbaumer, S., Fleury-Souverain, S., Antinori, P., Sadeghipour, F., Hochstrasser, D. F., Bonnabry, P., Veuthey, J-L., and Geiser, L. (2010). Simultaneous quantification of ten cytotoxic drugs by a validated LC–ESI–MS/MS method. *Analytical and Bioanalytical Chemistry, 398*(7), 3033-3042.

Ocampo-Pérez, R., Rivera-Utrilla, J., Mota, A. J., Sánchez-Polo, M., and Leyva-Ramos, R. (2016). Effect of radical peroxide promoters on the photodegradation of cytarabine antineoplastic in water. *Chemical Engineering Journal, 284*, 995-1002.

Ocampo-Pérez, R., Sánchez-Polo, M., Rivera-Utrilla, J., and Leyva-Ramos, R. (2010). Degradation of antineoplastic cytarabine in aqueous phase by advanced oxidation processes based on ultraviolet radiation. *Chemical Engineering Journal, 165*(2), 581-588.

Olicón-Hernández, D. R., González-López, J., and Aranda, E. (2017). Overview on the biochemical potential of filamentous fungi to degrade pharmaceutical compounds. *Frontiers in Microbiology, 8*, 1792.

Orias, F., and Perrodin, Y. (2013). Characterisation of the ecotoxicity of hospital effluents: a review. *Science of The Total Environment, 454*, 250-276.

Pandey, C. L., Maskey, G., Devkota, K., and Ojha, H. (2019). Investigating the institutional landscape for urban water security in Nepal. *Sustainability: The Journal of Record, 12*(3), 173-181.

Papinutti, V., Diorio, L., and Forchiassin, F. (2003). Production of laccase and manganese peroxidase by *Fomes sclerodermeus* grown on wheat bran. *Journal of Industrial Microbiology and Biotechnology, 30*(3), 157-160.

Parrella, A., Kundi, M., Lavorgna, M., Criscuolo, E., Russo, C., and Isidori, M. (2014). Toxicity of exposure to binary mixtures of four anti-neoplastic drugs in Daphnia magna and Ceriodaphnia dubia. *Aquatic Toxicology, 157*, 41-46.

Parrella, A., Lavorgna, M., Criscuolo, E., Russo, C., Fiumano, V., and Isidori, M. (2014). Acute and chronic toxicity of six anticancer drugs on *rotifers* and *crustaceans*. *Chemosphere, 115*, 59-66.

Paskulin, G. A., Gazzola Zen, P. R., de Camargo Pinto, L. L., Rosa, R., and Graziadio, C. (2005). Combined chemotherapy and teratogenicity. *Birth Defects Research Part A: Clinical and Molecular Teratology, 73*(9), 634-637.

Paudel, S., Kumar, P., Dasgupta, R., Johnson, B. A., Avtar, R., Shaw, R., Mishra, B. K., and Kanbara, S. (2021). Nexus between water security framework and public health: A comprehensive scientific review. *Water, 13*(10), 1365.

Pereira, C. S., Kelbert, M., Daronch, N. A., Michels, C., de Oliveira, D., and Soares, H. M. (2020). Potential of enzymatic process as an innovative technology to remove anticancer drugs in wastewater. *Applied Microbiology and Biotechnology, 104*(1), 23-31.

Pezzella, C., Macellaro, G., Sannia, G., Raganati, F., Olivieri, G., Marzocchella, A., Schlosser, D., and Piscitelli, A. (2017). Exploitation of Trametes versicolor for bioremediation of endocrine disrupting chemicals in bioreactors. *PloS One, 12*(6), e0178758.

Pieczyńska, A., Fiszka Borzyszkowska, A., Ofiarska, A., and Siedlecka, E. M. (2017). Removal of cytostatic drugs by AOPs: A review of applied processes in the context of green technology. *Critical Reviews in Environmental Science and Technology, 47*(14), 1282-1335.

Polovich, M., and Martin, S. (2011). Nurses use of hazardous drug-handling precautions and awareness of national safety guidelines. In *Oncology nursing forum, 38*(6).

Preston, B. L., and Snell, T. W. (2001). Full life-cycle toxicity assessment using *rotifer* resting egg production: Implications for ecological risk assessment. *Environmental Pollution, 114*(3), 399-406.

Prieto, A., Möder, M., Rodil, R., Adrian, L., and Marco-Urrea, E. (2011). Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. *Bioresource Technology, 102*(23), 10987-10995.

Pruijn, F. B., and DeWitte, R. S. (2004). A practical approach to improving drug transport in tumors. *Current Drug Discovery*, 29-32.

Qin, Z., Yue, Q., Borras-Hidalgo, O., and Liu, X. (2017). Laccase enzyme from *Trametes versicolor* with a high decolorizing ability on malachite green. *EC Microbiology*, *10*(3), 127-133.

Riva, F., Castiglioni, S., Fattore, E., Manenti, A., Davoli, E., and Zuccato, E. (2018). Monitoring emerging contaminants in the drinking water of Milan and assessment of the human risk. *International Journal of Hygiene and Environmental Health*, *221*(3), 451-457.

Roberts, P. H., and Thomas, K. V. (2006). The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Science of The Total Environment*, *356*(1-3), 143-153.

Rowney, N. C., Johnson, A. C., and Williams, R. J. (2009). Cytotoxic drugs in drinking water: a prediction and risk assessment exercise for the Thames catchment in the United Kingdom. *Environmental Toxicology and Chemistry*, *28*(12), 2733-2743.

Ruhí, A., Acuña, V., Barceló, D., Huerta, B., Mor, J.-R., Rodríguez-Mozaz, S., and Sabater, S. (2016). Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. *Science of The Total Environment, 540*, 250-259.

Russo, C., Lavorgna, M., Česen, M., Kosjek, T., Heath, E., and Isidori, M. (2018). Evaluation of acute and chronic ecotoxicity of cyclophosphamide, ifosfamide, their metabolites/transformation products and UV treated samples. *Environmental Pollution, 233*, 356-363.

Russo C., Lavorgna M., Piscitelli C., Isidori M. (2020) Toxicity of Anticancer Drug Residues in Organisms of the Freshwater Aquatic Chain. In: Heath E., Isidori M., Kosjek T., Filipič M. (eds). *Fate and Effects of Anticancer Drugs in the Environment*, (pp. 379-401), Springer, Cham.

Ryder, G. (2017). The United Nations world water development report, 2017: Wastewater: the untapped resource: UNESCO. (Source: https://stg-wedocs.unep.org/handle/20.500.11822/20448)

Sacher, F., Lange, F. T., Brauch, H.-J., and Blankenhorn, I. (2001). Pharmaceuticals in groundwaters: analytical methods and results of a monitoring program in Baden-Württemberg, Germany. *Journal of Chromatography A, 938*(1-2), 199-210.

Santana-Viera, S., Montesdeoca-Esponda, S., Sosa-Ferrera, Z., and Santana-Rodríguez, J. (2016). Cytostatic drugs in environmental samples: An update on the extraction and determination procedures. *TrAC Trends in Analytical Chemistry, 80*, 373-386.

Santos, L. H., Araújo, A. N., Fachini, A., Pena, A., Delerue-Matos, C., and Montenegro, M. (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials, 175*(1-3), 45-95.

Sato, M., Okada, Y., Oto, T., Minami, M., Shiraishi, T., Nagayasu, T., Ypshino, I., Chida, M., Okumura, M., Date, H., Miyoshi, S., and Kondo, T. (2014). Registry of the Japanese Society of Lung and Heart–Lung Transplantation: official Japanese lung transplantation report, 2014. *General Thoracic and Cardiovascular Surgery, 62*(10), 594-601.

Sebire, M., Allen, Y., Bersuder, P., and Katsiadaki, I. (2008). The model anti-androgen flutamide suppresses the expression of typical male stickleback reproductive behaviour. *Aquatic Toxicology, 90*(1), 37-47.

Seira, J., Sablayrolles, C., Montréjaud-Vignoles, M., Albasi, C., and Joannis-Cassan, C. (2016). Elimination of an anticancer drug (cyclophosphamide) by a membrane bioreactor: Comprehensive study of mechanisms. *Biochemical Engineering Journal, 114*, 155-163.

Singh, J., Saharan, V., Kumar, S., Gulati, P., and Kapoor, R. K. (2018). Laccase grafted membranes for advanced water filtration systems: a green approach to water purification technology. *Critical Reviews in Biotechnology, 38*(6), 883-901.

Šnajdr, J., & Baldrian, P. (2007). Temperature affects the production, activity and stability of ligninolytic enzymes in *Pleurotus ostreatus* and *Trametes versicolor*. *Folia Microbiologica, 52*(5), 498-502.

Sottani, C., Rinaldi, P., Leoni, E., Poggi, G., Teragni, C., Delmonte, A., and Minoia, C. (2008). Simultaneous determination of cyclophosphamide, ifosfamide, doxorubicin, epirubicin and daunorubicin in human urine using high-performance liquid chromatography/electrospray ionization tandem mass spectrometry: bioanalytical method validation. *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, *22*(17), 2645-2659.

Sottani, C., Tranfo, G., Faranda, P., and Minoia, C. (2005). Highly sensitive high-performance liquid chromatography/selective reaction monitoring mass spectrometry method for the determination of cyclophosphamide and ifosfamide in urine of health care workers exposed to antineoplastic agents. *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry, 19*(19), 2794-2800.

Steger-Hartmann, T., Kümmerer, K., and Hartmann, A. (1997). Biological degradation of cyclophosphamide and its occurrence in sewage water. *Ecotoxicology and Environmental Safety, 36*(2), 174-179.

Steger-Hartmann, T., Kümmerer, K., and Schecker, J. (1996). Trace analysis of the antineoplastics ifosfamide and cyclophosphamide in sewage water by twostep solid-

phase extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A, 726*(1-2), 179-184.

Straub, J. O. (2010). Combined environmental risk assessment for 5-fluorouracil and capecitabine in Europe. *Integrated Environmental Assessment and Management, 6*(S1), 540-566.

Sun, L., Zha, J., Spear, P. A., and Wang, Z. (2007). Toxicity of the aromatase inhibitor letrozole to Japanese medaka (Oryzias latipes) eggs, larvae and breeding adults. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 145*(4), 533-541.

Talbot, C. J., Bennett, E. M., Cassell, K., Hanes, D. M., Minor, E. C., Paerl, H., Raymond, P. R., Vargas, R., Vidon, P. G., Wollheim, W., and Xenopolous, M. A. (2018). The impact of flooding on aquatic ecosystem services. *Biogeochemistry, 141*(3), 439-461.

Tauxe-Wuersch, A., De Alencastro, L. F., Grandjean, D., and Tarradellas, J. (2005). Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. *Water Research, 39*(9), 1761-1772.

Ternes, T. A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. *Water Research, 32*(11), 3245-3260.

Ternes, T. A., Bonerz, M., Herrmann, N., Löffler, D., Keller, E., Lacida, B. B., and Alder, A. C. (2005). Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC tandem MS and GC/MS. *Journal of Chromatography A, 1067*(1-2), 213-223.

Toolaram, A. P., Kuemmerer, K., and Schneider, M. (2014). Environmental risk assessment of anti-cancer drugs and their transformation products: a focus on their genotoxicity characterization-state of knowledge and short comings. *Mutation Research/Reviews in Mutation Research, 760*, 18-35.

Tripathi, A. K., David, A., Govil, T., Rauniyar, S., Rathinam, N. K., Goh, K. M., and Sani, R. K. (2020). Environmental Remediation of Antineoplastic Drugs: Present Status, Challenges, and Future Directions. *Processes, 8*(7), 747.

Unicef. (2011). *UNICEF Annual Report 2010*: Unicef. (Source: Unicef - 2011 - books.google.com)

Vasiliadou, I., Sánchez-Vázquez, R., Molina, R., Martínez, F., Melero, J., Bautista, L., and Morales, G. (2016). Biological removal of pharmaceutical compounds using white-rot fungi with concomitant FAME production of the residual biomass. *Journal of Environmental Management, 180*, 228-237.

Verlicchi, P., Al Aukidy, M., Galletti, A., Petrovic, M., and Barceló, D. (2012). Hospital effluent: investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Science of The Total Environment, 430*, 109-118.

Verlicchi, P., Al Aukidy, M., and Zambello, E. (2015). What have we learned from worldwide experiences on the management and treatment of hospital effluent?—An overview and a discussion on perspectives. *Science of The Total Environment, 514*, 467-491.

Verlicchi P., Campos Garrigós A., Al Aukidy M. (2020). Occurrence of cytostatics in different water compartments. In: Heath E., Isidori M., Kosjek T., Filipič M. (eds). *Fate and Effects of Anticancer Drugs in the Environment*. (pp. 221-244), Springer, Cham.

Verliefde, A. R., Heijman, S., Cornelissen, E., Amy, G., Van der Bruggen, B., and Van Dijk, J. (2007). Influence of electrostatic interactions on the rejection with NF and assessment of the removal efficiency during NF/GAC treatment of pharmaceutically active compounds in surface water. *Water Research, 41*(15), 3227-3240.

Vidmar, J., Martinčič, A., Milačič, R., and Ščančar, J. (2015). Speciation of cisplatin in environmental water samples by hydrophilic interaction liquid chromatography coupled to inductively coupled plasma mass spectrometry. *Talanta, 138*, 1-7.

Vyas, N., Turner, A., and Sewell, G. (2014). Platinum-based anticancer drugs in waste waters of a major UK hospital and predicted concentrations in recipient surface waters. *Science of The Total Environment, 493*, 324-329.

Wang, J., Yamamoto, R., Yamamoto, Y., Tokumoto, T., Dong, J., Thomas, P., Hirai, H., and Kawagishi, H. (2013). Hydroxylation of bisphenol A by hyper lignin-degrading fungus *Phanerochaete sordida* YK-624 under non-ligninolytic condition. *Chemosphere, 93*(7), 1419-1423.

Wang, L., Albasi, C., Faucet-Marquis, V., Pfohl-Leszkowicz, A., Dorandeu, C., Marion, B., and Causserand, C. (2009). Cyclophosphamide removal from water by nanofiltration and reverse osmosis membrane. *Water Research, 43*(17), 4115-4122.

Wang, P., Hu, X., Cook, S., Begonia, M., Lee, K. S., & Hwang, H. M. (2008). Effect of culture conditions on the production of ligninolytic enzymes by white rot fungi *Phanerochaete chrysosporium* (ATCC 20696) and separation of its lignin peroxidase. *World Journal of Microbiology and Biotechnology*, *24*(10), 2205-2212.

Water, U. (2013). Water security and the global water agenda. *UN Water Analytical Brief. Hamilton, Canada: UN University*. (Source: https://www.unwater.org/publications/water-security-global-water-agenda)

Weissbrodt, D., Kovalova, L., Ort, C., Pazhepurackel, V., Moser, R., Hollender, J., Siegrist, H., and McArdell, C. S. (2009). Mass flows of X-ray contrast media and cytostatics in hospital wastewater. *Environmental Science and Technology, 43*(13), 4810-4817.

Wen, X., Jia, Y., and Li, J. (2010). Enzymatic degradation of tetracycline and oxytetracycline by crude manganese peroxidase prepared from Phanerochaete chrysosporium. *Journal of Hazardous Materials, 177*(1-3), 924-928.

Williams, T. D., Caunter, J. E., Lillicrap, A. D., Hutchinson, T. H., Gillings, E. G., and Duffell, S. (2007). Evaluation of the reproductive effects of tamoxifen citrate in partial and full life-cycle studies using fathead minnows (*Pimephales promelas*). *Environmental Toxicology and Chemistry: An International Journal*, *26*(4), 695-707.

Wormington, A. M., De María, M., Kurita, H. G., Bisesi, J. H., Denslow, N. D., and Martyniuk, C. J. (2020). Antineoplastic Agents: Environmental Prevalence and Adverse Outcomes in Aquatic Organisms. *Environmental Toxicology and Chemistry*.

Xiao, P., and Kondo, R. (2020). Biodegradation and biotransformation of pentachlorophenol by wood-decaying white rot fungus *Phlebia acanthocystis* TMIC34875. *Journal of Wood Science, 66*(1), 1-11.

Xie, Z., Lu, G., Yan, Z., Liu, J., Wang, P., and Wang, Y. (2017). Bioaccumulation and trophic transfer of pharmaceuticals in food webs from a large freshwater lake. *Environmental Pollution, 222*, 356-366.

Yin, J., Shao, B., Zhang, J., and Li, K. (2010). A preliminary study on the occurrence of cytostatic drugs in hospital effluents in Beijing, China. *Bulletin of Environmental Contamination and Toxicology*, *84*(1), 39.

Zhang, J., Chang, V. W., Giannis, A., and Wang, J.Y. (2013a). Removal of cytostatic drugs from aquatic environment: a review. *Science of The Total Environment, 445*, 281-298.

Zhang, J., Chang, V. W. C., Giannis, A., and Wang, J.-Y. (2013b). Removal of cytostatic drugs from aquatic environment: A review. *Science of The Total Environment, 445-446*, 281-298.

Zhang, T., Cai, L., Xu, B., Li, X., Qiu, W., Fu, C., & Zheng, C. (2019). Sulfadiazine biodegradation by *Phanerochaete chrysosporium*: Mechanism and degradation product identification. *Chemosphere, 237*, 124418.

Zhang, Y., and Geißen, S.-U. (2010). In vitro degradation of carbamazepine and diclofenac by crude lignin peroxidase. *Journal of Hazardous Materials*, *176*(1-3), 1089-1092.

Zounkova, R., Kovalova, L., Blaha, L., and Dott, W. (2010). Ecotoxicity and genotoxicity assessment of cytotoxic antineoplastic drugs and their metabolites. *Chemosphere, 81*(2), 253-260.

Zounková, R., Odráška, P., Doležalová, L., Hilscherová, K., Maršálek, B., and Bláha, L. (2007). Ecotoxicity and genotoxicity assessment of cytostatic pharmaceuticals. *Environmental Toxicology and Chemistry: An International Journal, 26*(10), 2208-2214.

Publications

Research/Review article

1. **Yadav, A**., Rene, E. R., Mandal, M. K., and Dubey, K. K. (2021). Biodegradation of cyclophosphamide and etoposide by white-rot-fungi and their degradation kinetics *Bioresource Technology*, 126355. **(Impact Factor- 9.64**)

2. **Yadav, A**., Rene, E. R., Mandal, M. K., and Dubey, K. K. (2021). Threat and sustainable technological solution for antineoplastic drugs pollution: Review on a persisting global issue. *Chemosphere*, *263*, 128285. (**Impact Factor- 7.086**)

3. Cristóvão, M. B., Janssens, R., **Yadav, A**., Pandey, S., Luis, P., Van der Bruggen, B., Dubey, K. K., Mandal, M. K., Crespo, J. G., and Pereira, V. J. (2020). Predicted concentrations of anticancer drugs in the aquatic environment: What should we monitor and where should we treat? *Journal of hazardous materials*, *392*, 122330. **(Impact Factor- 10.558**)

4. **Yadav, A**., Mandal, M. K., and Dubey, K. K. (2020). In Vitro Cytotoxicity Study of Cyclophosphamide, Etoposide and Paclitaxel on Monocyte Macrophage Cell Line Raw 264.7. *Indian Journal of Microbiology*, *60*(4), 511-517. **(Impact Factor- 2.46)**

5. **Yadav, A**., Pandey, S., Mandal, M. K., and Dubey, K. K. (2020). Development of cost-effective RP-HPLC methods for detection of cyclophosphamide, etoposide and paclitaxel. *Separation Science Plus*, *3*(3), 40-43.

6. Badhwar, P., Kumar, A., **Yadav, A**., Kumar, P., Siwach, R., Chhabra, D., and Dubey, K. K. (2020). Improved pullulan production and process optimization using novel GA–ANN and GA–ANFIS hybrid statistical tools. *Biomolecules*, *10*(1), 124. **(Impact Factor- 4.87)**

7. Goyal, M., Chauhan, S., **Ankush**., Goyal, P., and Prabha, J. (2018). Structural modelling of shikimate pathway enzymes for herbicide and drug development: A review. *Journal of Entomology and Zoology Studies*, *6*(2), 785-790.

8. **Journal Cover Page Image**: **Yadav, A**., Pandey, S., Mandal, M. K., & Dubey, K. K. (2020). Development of cost-effective RP-HPLC methods for detection of cyclophosphamide, etoposide and paclitaxel. *Separation Science Plus*, *3*(3), 40-43.

Book chapter

1. **Ankush**., Mandal, M. K., Sharma, M., Khushboo., Pandey, S., and Dubey, K. K. (2019). Membrane technologies for the treatment of pharmaceutical industry wastewater. In *Water and Wastewater Treatment Technologies* (pp. 103-116). Springer, Singapore. (ISBN No. 9789811332593)

2. **Ankush**., Khushboo., and Dubey, K. K. (2020). Food industry waste biorefineries: future energy, valuable recovery, and waste treatment. In *Refining Biomass Residues for Sustainable Energy and Bioproducts* (pp. 391-406). Academic Press. (ISBN No. 9780128189979)

3. Sharma, M., **Yadav, A**., Mandal, M. K., Pandey, S., Pal, S., Chaudhuri, H., Chakrabarti, S., and Dubey, K. K. (2021). Wastewater treatment and sludge management strategies for environmental sustainability. In *Circular Economy and Sustainability* (pp. 97-112). Elsevier. (ISBN No. 9780128216644)

4. Goyal, M., **Ankush**., Jangra, M.R., Batra, R., and Kumar, P. (2019). Aptamer-Based Biosensors for Detection of Environmental Pollutants. In *Aptamers* (pp. 155-167). Springer, Singapore. (ISBN No. 9789811388354)

5. Indu., **Yadav, A**., Mandal, M. K., and Dubey, K. K. (2020). Nanomaterial Biosynthesis and Enzyme Immobilization: Methods and Applications. *Green Synthesis of Nanomaterials for Bioenergy Applications* (pp.191-209). John Wiley & Sons. (ISBN No. 9781119576785)

6. Khushboo., **Ankush**., Yadav, K., Mandal, M. K., Pal, S., Chaudhuri, H., and Dubey, K. K. (2020). Bioeconomy of municipal solid waste (MSW) using gas fermentation. In *Current Developments in Biotechnology and Bioengineering* (pp. 289-304). Elsevier. (ISBN No. 9780444643216)

7. Dubey, K. K., Pramanik, A., **Ankush**., Khushboo., and Yadav, J. (2019). Enzyme Engineering. In *Advances in Enzyme Technology* (pp. 325-347). Elsevier. (ISBN No. 9780444641144)

8. Yadav, J., **Ankush**., Khushboo., Thakur, M., Yadav, K., Sharma, M., and Dubey, K. K. (2019). Aptasensor-Possible Design and Strategy for Aptamer Based Sensor. In *Aptamers* (pp. 133-154). Springer, Singapore. (ISBN No. 9789811388354)

Patent

1. Published an Indian Patent on "A Paper Strip based Test for Detection of Aminoglycosides Group of Antibiotics in Water and Wastewater" (Application No. 2017110044714).

2. Published an Indian Patent on "A Rapid Method for Detection of Ammonium Sulphate Adulteration in Milk" (Application No. 201811026150).

3. Published an Indian Patent on "A Rapid Method for Detection of Urea in Milk and Potable Water" (Application No. 201911027350).

4. Published an Indian Patent on "A Strip based Method for Detection of Urea in Milk" (Application No. 201911027351).

Conference paper

1. **Ankush**, Kashyap Kumar Dubey "Genotoxic activity detection of cytostatic compounds cyclophosphamide, etoposide and paclitaxel in hospital wastewater" in International conference on NHBT-New Horizons in Biotechnology, 20-24th November 2019, CSIR-NIIST, Trivandrum, India. (**Poster Presentation**)

2. **Ankush,** Kashyap Kumar Dubey "Cytostatic drugs in aquatic environment and their detection by RP-HPLC method" in 60th Annual Conference of Association of Microbiologists of India (AMI-2019) and International Symposium on "Microbial Technologies in Sustainable Development of Energy, Environment Agriculture and Health" 15-18th November 2019, CUH Mahendergarh, India. **(Poster Presentation**)

3. Kashyap Kumar Dubey, **Ankush**, Mrinal Kanti Mandal, J.G. Crespo, Patricia Luis, "Strategies for treatment of Hospital Wastewater using Integrated Technology" in BRICS-TEQIP Sponsored workshop on Recent trends on remediation of contaminated water bodies and soil, 25-29th March 2019, NIT Durgapur, West-Bengal, India. (**Oral Presentation)**

4. **Ankush,** Kashyap Kumar Dubey "Occurrence and Risk assessment of Anticancer Compound (Cyclophosphamide and Etoposide) in Environment" in International Conference on Bio-innovation for Environmental and Health Sustainable Developments (BEHSD)-2018, 27-28th November 2018, CSIR-IITR Lucknow, India. **(Poster Presentation)**

5. **Ankush,** Kashyap Kumar Dubey "HPLC method development for analysis of Cyclophosphamide, Paclitaxel and Etoposide and study mutagenic effect on *Salmonella typhimurium*" in International Conference on The 6th IWA Regional Membrane

Technology Conference (IWA-RMTC)-2018, 10-12th December 2018, MSU Vadodara, India. **(Poster Presentation)**

6. **Ankush,** Khushboo, Karuna Yadav, Kashyap Kumar Dubey**, "**Barapullah drain Water Treatment with *Chlorella vulgaris*" in National Conference on Biotechnology: Exploring Through Innovations, 13-14th November 2017, CUH Mahendergarh, India. **(Poster Presentation)**

Bioresource Technology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biodegradation of cyclophosphamide and etoposide by white rot fungi and their degradation kinetics

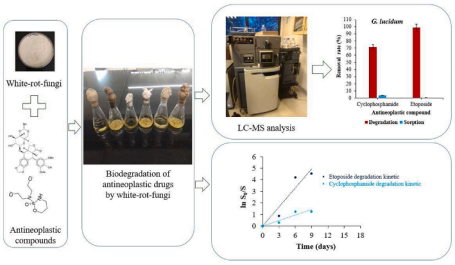
Ankush Yadav [a], Eldon R. Rene [b], Mrinal Kanti Mandal [c], Kashyap Kumar Dubey [d,*]

[a] Department of Biotechnology, Central University of Haryana, Mahendergarh 123031, India
[b] Department of Water Supply, Sanitation and Environmental Engineering, IHE Delft Institute for Water Education, Westvest 7, 2611AX Delft, the Netherlands
[c] Department of Chemical Engineering, National Institute of Technology, Durgapur 713209, India
[d] Bioprocess Engineering Laboratory, School of Biotechnology, Jawaharlal Nehru University, New Delhi 110067, India

HIGHLIGHTS

- Degradation of antineoplastic compounds by white rot fungi were evaluated.
- *Ganoderma lucidum* showed the highest degradation for cyclophosphamide and etoposide.
- Cyclophosphamide showed highest sorption efficiency with *P. chrysosporium.*
- Maximum degradation was achieved for etoposide ($k = 0.54$ day^{-1}).

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cytostatic compounds
Etoposide
WRFs
Treatment
Kinetics

ABSTRACT

The biodegradation of cyclophosphamide and etoposide by *Trametes versicolor* (AH05), *Ganoderma lucidum* (MTCC-1039), and *Phanerochaete chrysosporium* (MTCC-787) were tested for 3, 6, 9, 12, and 15 days, respectively. *G. lucidum* achieved the highest degradation efficiency of cyclophosphamide (71.5%) and etoposide (98.4%) after 6 days of treatment. The degradation efficiency of *T. versicolor* and *P. chrysosporium* for etoposide was 79.8% and 76.8%, respectively. However, no degradation of cyclophosphamide was achieved with *P. chrysosporium*, although it showed the highest sorption efficiency for cyclophosphamide (23.7%). *Trametes versicolor* achieved only 1.4% degradation of cyclophosphamide, that includes both biodegradation and biosorption. The pseudo first-order degradation kinetics explained the degradation of etoposide and cyclophosphamide with $t_{1/2}$ values of 1.32 and 4.43 days and '*k*' constant of 0.16 and 0.54 day^{-1}, respectively.

1. Introduction

Cancer is the 2nd highest non-communicable global leading disease (Bray et al., 2018). To protect from this disease, antineoplastic compounds are designed to arrest the cell proliferation process by inhibiting DNA replication during the cell cycle and block rapid cell division. Antineoplastic compounds have various detrimental effects and potential to cause harmful effects on aquatic life as well as human health (Yadav et al., 2021). Several reports have indicated that all the exposed animals showed symptoms of some abnormality in physiological/physiochemical behaviour, reproductive change, zygote development which can cause an imbalance of genetic material and leads to cell death or

* Corresponding Author: School of Biotechnology, Jawaharlal Nehru University, New Delhi 110067, India.
E-mail address: ashyapdubey@gmail.com (K. Kumar Dubey).

https://doi.org/10.1016/j.biortech.2021.126355
Received 31 August 2021; Received in revised form 9 November 2021; Accepted 12 November 2021
Available online 17 November 2021

Chemosphere 263 (2021) 128285

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Threat and sustainable technological solution for antineoplastic drugs pollution: Review on a persisting global issue

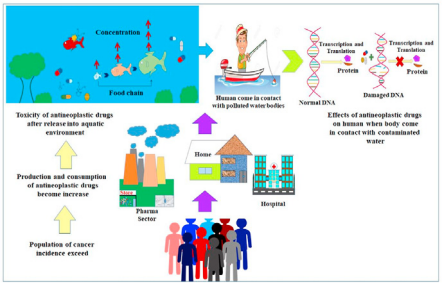
Ankush Yadav [a], Eldon R. Rene [b], Mrinal Kanti Mandal [c], Kashyap Kumar Dubey [a, d, *]

[a] Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh, 123031, Haryana, India
[b] Department of Water Supply, Sanitation and Environmental Engineering, IHE Delft Institute for Water Education, Westvest 7, 2611AX, Delft, the Netherlands
[c] Department of Chemical Engineering, NIT Durgapur, Durgapur, 713209, West Bengal, India
[d] Bioprocess Engineering Laboratory, School of Biotechnology, Jawaharlal Nehru University, New Delhi, 110067, India

HIGHLIGHTS

- Role of antineoplastic agents as emerging water contaminants has been discussed.
- The health implications of antineoplastic compounds were reviewed.
- Strategies for the treatment of antineoplastic compounds were analyzed.
- The role of stringent discharge regulations and its implementation were identified.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 April 2020
Received in revised form
31 August 2020
Accepted 6 September 2020
Available online 8 September 2020

Handling Editor: Derek Muir

Keywords:
Antineoplastic
Wastewater
Cyclophosphamide
Toxicity
Food chain
Treatment

ABSTRACT

In the past 20 years, the discharge of pharmaceuticals and their presence in the aquatic environment have been continuously increasing and this has caused serious public health and environmental concerns. Antineoplastic drugs are used in chemotherapy, in large quantities worldwide, for the treatment of continuously increasing cancer cases. Antineoplastic drugs also contaminate water sources and possess mutagenic, cytostatic and eco-toxicological effects on microorganisms present in the aquatic environment as well as on human health. Due to the recalcitrant nature of antineoplastic drugs, the commonly used wastewater treatment processes are not able to eliminate these drugs. Globally, various anticancer drugs are being consumed during chemotherapy in hospitals and households by out-patients. These anticancer agents enter the water bodies in their original form or as metabolites via urine and faeces of the out-patients or the patients admitted in hospitals. Due to its high lipid solubility, the antineoplastic drugs accumulate in the fatty tissues of the organisms. These drugs enter through the food chain and cause adverse health effects on humans due to their cytotoxic and genotoxic properties. The United States Environmental Protection Agency (US-EPA) and the Organization for Economic Cooperation and Development (OECD) elucidated new regulations for the management of hazardous pharmaceuticals in the water environment. In this paper, the role of antineoplastic agents as emerging water contaminants, its transfer through the food chain, its eco-toxicological properties and effects, technological solutions and management aspects were reviewed.

* Corresponding author. Bioprocess Engineering Laboratory, School of Biotechnology, Jawaharlal Nehru University, New Delhi, 110067, India.
E-mail address: kashyapdubey@gmail.com (K.K. Dubey).

https://doi.org/10.1016/j.chemosphere.2020.128285
0045-6535/

Journal of Hazardous Materials 392 (2020) 122330

Contents lists available at ScienceDirect

Journal of Hazardous Materials

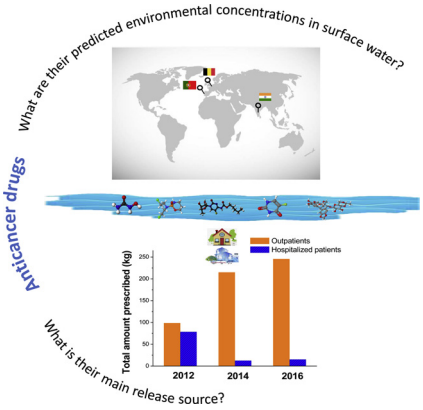
journal homepage: www.elsevier.com/locate/jhazmat

Predicted concentrations of anticancer drugs in the aquatic environment: What should we monitor and where should we treat?

M.B. Cristóvão[a,f], R. Janssens[b], A. Yadav[c], S. Pandey[d], P. Luis[b], B. Van der Bruggen[e], K.K. Dubey[c], M.K. Mandal[d], J.G. Crespo[f], V.J. Pereira[a,g,*]

[a] iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
[b] Materials and Process Engineering, UCLouvain, Louvain-la-Neuve, Belgium
[c] Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh, 123031, Haryana, India
[d] National Institute of Technology Durgapur, M.G. Avenue, Durgapur, West Bengal, India
[e] KULeuven, Leuven, Belgium
[f] LAQV-REQUIMTE/Department of Chemistry, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal
[g] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: D. Aga

Keywords:
Anticancer drugs
Consumption pattern
Hospitalized and outpatients
Entry route
Predicted environmental concentrations

ABSTRACT

Anticancer drugs have been detected in the aquatic environment, they have a potent mechanism of action and their consumption is expected to drastically increase in the future. Consequently, it is crucial to routinely monitor the occurrence of anticancer drugs and to develop effective treatment options to avoid their release into the environment.

Prior to implementing a monitoring program, it is important to define which anticancer drugs are more prone to be found in the surface waters. In this study the consumption of anticancer drugs in the Lisbon region (Portugal), Belgium and Haryana state (India) were used to estimate the concentrations that can be expected in surface waters.

Moreover, one important aspect is to define the major entry route of anticancer drugs in the aquatic environment: is it hospital or household effluents? The results disclosed in this study showed that in Belgium and Lisbon, 94 % of the total amount of anticancer drugs were delivered to outpatients, indicating that household

* Corresponding author.
E-mail address: vanessap@ibet.pt (V.J. Pereira).

https://doi.org/10.1016/j.jhazmat.2020.122330
Received 25 November 2019; Received in revised form 5 February 2020; Accepted 15 February 2020
Available online 19 February 2020

Indian J Microbiol
https://doi.org/10.1007/s12088-020-00896-1

ORIGINAL RESEARCH ARTICLE

In Vitro Cytotoxicity Study of Cyclophosphamide, Etoposide and Paclitaxel on Monocyte Macrophage Cell Line Raw 264.7

Ankush Yadav[1] · Mrinal Kanti Mandal[2] · Kashyap Kumar Dubey[1]

Received: 6 March 2020 / Accepted: 11 June 2020

Abstract The presence of antineoplastic compounds in aquatic ecosystem is an emerging challenge for the society. Antineoplastic compounds released into the aquatic environment exhibit a potential threat to normal aquatic life. Particularly, antineoplastic compounds are responsible for direct or indirect interference with the cellular DNA of an organism and cause toxicity to cells. The present study focused on the assessment of in vitro toxic effect of cyclophosphamide, etoposide and paclitaxel on Raw 264.7 cell line (mouse monocyte macrophage cells). The inhibitory concentration of cyclophosphamide, etoposide, and paclitaxel was determined. The IC_{50} values of these compounds were 145.44, 5.40, and 69.76 μg ml^{-1} respectively. This is the first report on toxicity analysis of cyclophosphamide, paclitaxel and etoposide on Raw 264.7 cell line by reducing cell viability and indicating the cell cytotoxicity i.e., 69.58% for cyclophosphamide, 92.01% for etoposide and 88.85% for paclitaxel on concentration 250 μg ml^{-1}. The results of their cytotoxicity assessment highlight the need of improvement in sewage treatment technology for the efficient removal of these compounds from aquatic environment.

Keywords Anticancer compounds · Cell culture · Immune cells · Toxicity · Cell viability

✉ Kashyap Kumar Dubey
kashyapdubey@gmail.com

[1] Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana 123031, India

[2] Department of Chemical Engineering, National Institute of Technology Durgapur, Durgapur, West-Bengal 713029, India

Introduction

Worldwide, cancer is the second highest non-communicable disease after cardiovascular disease. The incidence of new cancer cases in year 2012 was 14.1 million and it becomes increases to 18.07 million in the year 2018 [1]. Consequently, this increment in cancer incidence leads to the demand, production and consumption of antineoplastic drugs [2–4]. Unexpectedly, through the oncology wards of hospitals, discharge of hospitalized patients, outpatients and due to lack of treatment facility in STPs (sewage treatment plant), antineoplastic compounds are persistently coming into water bodies. Several studies investigated the presence of these compounds in aquatic the environment and the occurrence is due to persistence or recalcitrant nature of antineoplastic drugs after going through treatment plants and remain dynamic after pass through wastewater treatment plant [5–11]. Antineoplastic drugs are non-specific in nature and have a property to kill or inhibit cell growth by blocking the cell cycle. So, due to their lack of specificity and negative interaction with cellular DNA, they are cytostatic and mutagenic for normal cells even present at very low concentrations in water bodies [12].

Researchers reported the toxicity (cytotoxicity, mutagenicity, and ecotoxicity) of antineoplastic compounds on different models in terms of EC_{50} (effective concentration), LC_{50} (median lethal dose), IC_{50} (inhibitory concentration), LOEC (Lowest observed effect concentration), and NOEC (No observed effect concentration) [13–17]. But the effect of cyclophosphamide, etoposide and paclitaxel on the immune system of any organism is not elucidated yet. Every organism has a defence mechanism against pathogens and other toxic substances [18, 19]. The immune system has different specialized cells which protect the body from harmful substance. Among these cells, the

Published online: 18 June 2020

Received: 27 August 2019 | Revised: 7 December 2019 | Accepted: 9 December 2019

DOI: 10.1002/sscp.201900071

SHORT COMMUNICATION

Development of cost-effective RP-HPLC methods for detection of cyclophosphamide, etoposide and paclitaxel

Ankush Yadav[1] | **Shailesh Pandey**[2] | **Mrinal Kanti Mandal**[2] | **Kashyap Kumar Dubey**[1]

[1]Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh-123031

[2]Department of Chemical Engineering, National Institute of Technology Durgapur, West-Bengal-713209

Correspondence
Dr. Kashyap Kumar Dubey, Department of Biotechnology, Central University of Haryana, Mahendergarh-123031.
Email: kashyapdubey@gmail.com

Funding information
Department of Biotechnology , Ministry of Science and Technology, Grant/Award Number: BT/IN/INNO-INDIGO/26/MKM/2015-16

Quantification of cyclophosphamide, palcitaxel and etoposide in water sample using simple analytical technique is challenging. In this study, we have developed a simple, sensitive, robust and accurate method for the detection and quantification of cyclophosphamide, etoposide, and paclitaxel using high-performance liquid chromatography and validated the same. The analytical conditions were achieved by the high-performance liquid chromatography system on a C_{18} column (250 mm × 4.6 mm × 5 μm) under isocratic mode. The retention time for cyclophosphamide, paclitaxel and etoposide were 2:40.1, 3:32.3 and 3:49.3 min respectively. We validated the method by evaluating system sensitivity and linearity. We anticipate the method to be useful in quantification of cyclophosphamide, paclitaxel and etoposide in water sample.

KEYWORDS
cytostatic compounds, method standardization, validation

1 | INTRODUCTION

In today's world, the population of cancer patients is increasing day by day [1,2], consequently the consumption rates of cytostatic drugs are also increasing. Cytostatic compounds are delivered into the environment from different sources such as hospital effluent and municipal effluent of outpatients [3–6]. The occurrence of cytostatic compound and other pharmaceutical drugs have been widely reported in the aqueous environment [5,7–12]. Cytostatic compound's presence in the environment is a potential threat [13]. The major cytostatic compounds that are detected in the environmental water samples are cyclophosphamide, paclitaxel, and etoposide (REF). Cyclophosphamide is an alkylating agent (MW-261.086), Paclitaxel is a plant alkaloid (MW-358.906) and Etoposide (MW-588.557) is a topoisomerase inhibitor that is used widely at a very higher rate (kg/year) in the hospitals for treatment of cancer patients [2,8]. Presence of these compounds in aqueous environment poses severe threat and may even cause genetic diseases upon consumption. Therefore it is critical to detect their presence and to quantify them in environmental samples [12].

Although there are several literatures on individual or simultaneous detection of paclitaxel, cyclophosphamide and etoposide [2,8,14–17], these reported methods usually require very costly solvent as a mobile phase. Therefore, there is a need for simple and low cost technique to detect these compounds.

The aim of present study is to develop a simple, sensitive, robust and reliable RP-HPLC analytical method for the determination and quantification of cyclophosphamide, etoposide and paclitaxel in environmental water samples.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagent

HPLC grade ACN and water was procured from Merck, cytostatic compound Cyclophosphamide was purchased from Hi-Media (RM8152), Etoposide from TCI (E0675), Paclitaxel from Hi-media (RM9750), and 0.2 μm syringe filter used in this study is from axiva (SFNY13RB).

Article Related Abbreviation: RT, Retention time.

biomolecules

MDPI

Article

Improved Pullulan Production and Process Optimization Using Novel GA–ANN and GA–ANFIS Hybrid Statistical Tools

Parul Badhwar [1,†], Ashwani Kumar [2,†], Ankush Yadav [3,†], Punit Kumar [1], Ritu Siwach [1], Deepak Chhabra [2] and Kashyap Kumar Dubey [3,*]

1 Microbial Process Development Laboratory, University Institute of Engineering and Technology, Maharishi Dayanand University, Rohtak-124001, Haryana, India; b6parul@gmail.com (P.B.); punitdariyapur@gmail.com (P.K.); ritusiwach91@gmail.com (R.S.)

2 Optimization and Mechatronics Laboratory, Department of Mechanical Engineering, University Institute of Engineering and Technology, Maharishi Dayanand University, Rohtak-124001, Haryana, India; goswamiashwanikumar@gmail.com (A.K.); deepak.chhabra@mdurohtak.ac.in (D.C.)

3 Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh-123031, Haryana, India; ankushrao540@gmail.com

* Correspondence: kashyapdubey@cuh.ac.in; Tel.: +919996122280

† These authors contributed equally to this work.

Received: 19 December 2019; Accepted: 8 January 2020; Published: 10 January 2020

Abstract: Pullulan production from *Aureobasidium pullulans* was explored to increase yield. Non-linear hybrid mathematical tools for optimization of process variables as well as the pullulan yield were analyzed. The one variable at a time (OVAT) approach was used to optimize the maximum pullulan yield of 35.16 ± 0.29 g/L. The tools predicted maximum pullulan yields of 39.4918 g/L (genetic algorithm coupled with artificial neural network (GA–ANN)) and 36.0788 g/L (GA coupled with adaptive network based fuzzy inference system (GA–ANFIS)). The best regression value (0.94799) of the Levenberg–Marquardt (LM) algorithm for ANN and the epoch error (6.1055×10^{-5}) for GA–ANFIS point towards prediction precision and potentiality of data training models. The process parameters provided by both the tools corresponding to their predicted yield were revalidated by experiments. Among the two of them GA–ANFIS results were replicated with 98.82% accuracy. Thus GA–ANFIS predicted an optimum pullulan yield of 36.0788 g/L with a substrate concentration of 49.94 g/L, incubation period of 182.39 h, temperature of 27.41 °C, pH of 6.99, and agitation speed of 190.08 rpm.

Keywords: Pullulan; genetic algorithm; artificial neural network; fermentation

1. Introduction

Pullulan is a biopolymer of high commercial importance and utility [1]. Pullulan which is mostly available in powdered form, can also be formulated to thin films. These films have numerous applications in food, pharma, and healthcare industries. The most popular and commercially successful application of pullulan films is in Listerine® mouth freshener. Pullulan has also received wider acceptance in the food sector, and due to its intensifying nature has become an accepted ingredient in soups, sauces, and beverages [2]. Pullulan can be used in pharmaceuticals, such as in coatings on pills and capsules, including sustained-release formulations [2].

For fermentative production of pullulan, *Aureobasidium pullulans* is the preferred microbial source. Pullulan as an exopolysaccharide is produced by *A. pullulans* in response to external pH change and nutrient deficiency [3]. *A. pullulans*, as well as its upstream processing, has been the subject of an increasing body of research in fermentation studies. For an efficacious full fermenter system analysis,

Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 785-790

Received: 03-01-2018
Accepted: 04-02-2018

Meenu Goyal
Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India

Sugandh Chauhan
Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India

Ankush
Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India

Preeti Goyal
Faculty of Life Sciences, PDM University, Bahadurgarh, Haryana, India

Jyoti Prabha Bishnoi
Amity institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

Correspondence
Jyoti Prabha Bishnoi
Amity institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India

Structural modeling of shikimate pathway enzymes for herbicide and drug development: A review

Meenu Goyal, Sugandh Chauhan, Ankush, Preeti Goyal and Jyoti Prabha Bishnoi

Abstract
The shikimate pathway is the biosynthetic route for aromatic amino acids in microbes and plants but not in animals. Due to the absence of this pathway in animals, it is the main target for action of herbicides and antimicrobial agents. All the enzymes of this pathway have been targeted for herbicide and drug development. The EPSP (5-enolpyruvylshikimate-3-phosphate) synthase is one of the important enzymes of the shikimate pathway which is also involved in the biosynthesis of various secondary metabolites essential for survival. DAHP (3-Deoxy-D-arabinoheptulosonate 7-phosphate synthase) is the first enzyme of this pathway, which is involved in the condensation of PEP (Phosphoenolpyruvate) and E4P (D- erythrose 4-phosphate) to produce DAHP. Chorismate synthase and shikimate kinase are other enzyme targets of the pathway. To develop new herbicides and drugs targeting this pathway, three dimentional (3D) structure of the target enzymes must be known. But still a large number of protein structures are not available due to difficulties in wet lab determination of protein structures. This review highlights the importance of computational techniques for structural modeling of enzymes of shikimate pathway and subsequent applications for developing new herbicides and drugs.

Keywords: shikimate, herbicide, modeling

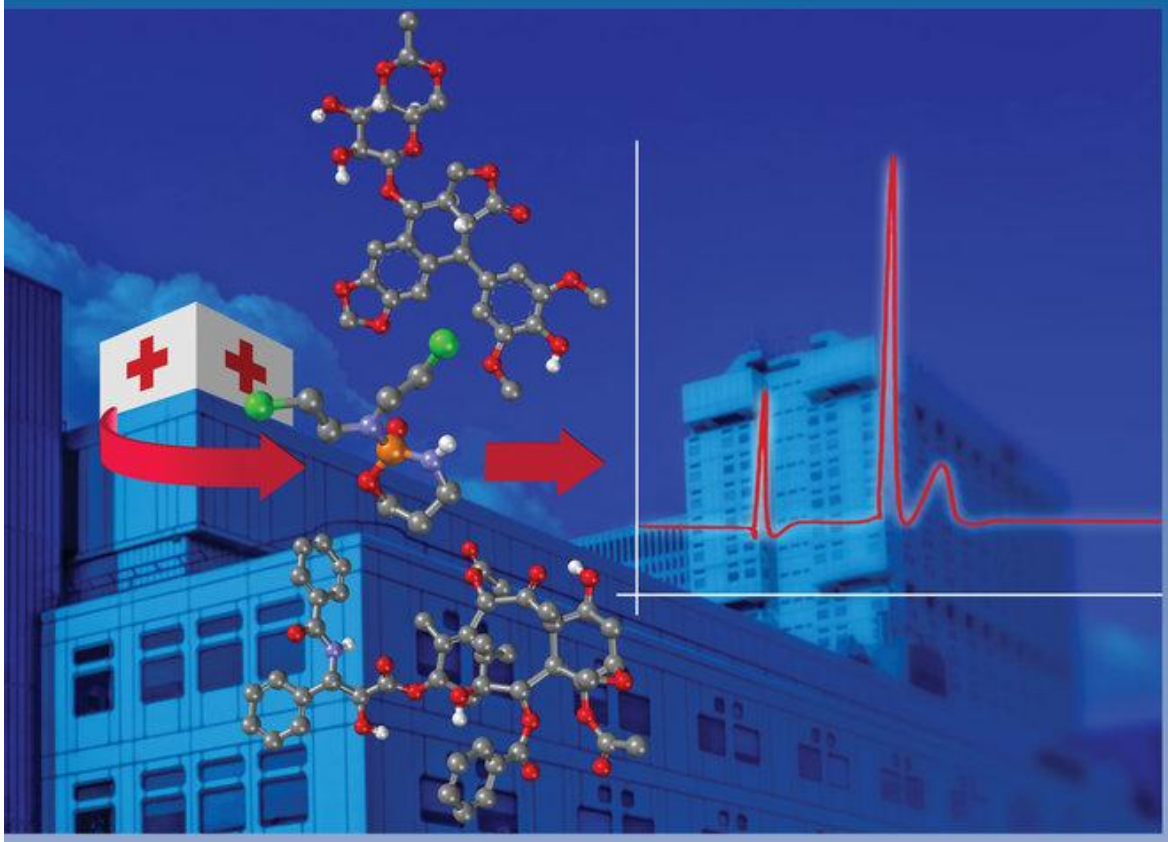
Introduction
Due to exponential increase in the rate of protein sequencing, more than one million protein sequences are available in the sequence databases (SwissProt, Protein Information Resource). The primary database for protein structure information is the Protein Data Bank (PDB) [1]. The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) provides an information for structures of biological macromolecules. Various tools and resources would required for understanding the relationship between function of biological macromolecules, sequence and structure [2]. The prediction of the 3D structure of a protein sequence is a difficult task. Wet lab techniques for determination of protein structure by X-ray crystallography or Nuclear Magnetic Resonance (NMR) is expensive and complex. Therefore, protein threading, homology modeling and ab initio were developed under computational methods and the most simple and reliable method is homology modeling. The goal of protein modeling is to predict the structure of a protein from its amino acid with an accuracy that is comparable to the best results achieved experimentally [3]. Homology modeling refers to construct a protein 3D structure using an already existing experimentally determined structure that closely relating at the sequence level [4]. 3D structure of a given protein is predicted via homology modeling based on its alignment to one or more proteins of known structure (templates).The prediction process includes identification of template, target-template alignment, model building, model refinement and model validation. There are several computer programs and Web servers that may be used for modeling [5]. The sequence with similarity greater than 30% can act as template [6]. Threading method compare a target sequence against a library of structural templates, producing a list of scores. According to the rank, the best fold is assumed to be one adopted by the sequence. *Ab initio* prediction method assumes that the native structure of a protein is at the Gibbs free energy minimum [7].

Shikimate Pathway
The shikimate pathway is an important and common pathway for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan and also serve as precursors

ISSN 2573-1815 · SSC 3 (3) 37–72 (2020) · Vol. 3 · No. 3 · March 2020

SSC plus

SEPARATION SCIENCE PLUS



Methods
Chromatography · Electroseparation

Applications
Biomedicine · Foods · Environment

3|20

www.sscp-journal.com

WILEY-VCH

Chapter 6
Membrane Technologies for the Treatment of Pharmaceutical Industry Wastewater

Ankush, Mrinal Kanti Mandal, Manisha Sharma, Khushboo, Shailesh Pandey and Kashyap Kumar Dubey

Abstract In course of past few years, pharmaceutical industries have huge contribution in the economic development of the country, but concurrently the pharmaceutical pollutants can also be responsible for severe hazards to the environment. Traditional methods of wastewater treatment cannot erase these pollutants from the water due to their hostile behavior. The advent of the pharmaceutical pollutants leads a demand for assessment and depiction of the wastewater discharged from the pharmaceutical industry as per the norms recommended by the official agency (Pollution Control Board). Vast number of treatment strategies are adapted by the pharmaceutical industries to reuse wastewater and regulate environmental pollution. In this chapter, we mainly focus on the finest membrane based methodologies to abolish the pharmaceutical compounds. At present, no individual technology has the potential to expel out the pharmaceutical pollutants from wastewater. Merging of traditional methods with membrane reactors leads to the best hybrid wastewater treatment technology.

Keywords Pharmaceutical pollutant · Reverse osmosis · Nanofiltration Microfiltration · Ultrafiltration and MBR

6.1 Introduction

In the present days, the disease burdens are increasing due to anti-microbial resistance (AMC), with regards to this the market of antibiotics, drugs, and other pharmaceutical compounds are also growing. So, the release of these pharmaceu-

Ankush · M. Sharma · Khushboo · K. K. Dubey (✉)
Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh 123031, Haryana, India
e-mail: kashyapdubey@gmail.com

M. K. Mandal · S. Pandey
Department of Chemical Engineering, National Institute of Technology, Durgapur 713209, West Bengal, India

X.-T. Bui et al. (eds.), *Water and Wastewater Treatment Technologies*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-13-3259-3_6

Food industry waste biorefineries: future energy, valuable recovery, and waste treatment

17

Ankush, Khushboo and Kashyap Kumar Dubey
Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh, India

17.1 Introduction

The population is growing at a very fast rate, which indirectly increases the demand of energy, chemicals, food, and other important things required for survival. The increasing demand of the society is pushing the researchers to introduce novel techniques with low adverse effect on the environment. An alternative feedstock of renewable raw materials over the fossil-based raw material admires the scientists to create the concept of biorefinery. The process of biorefinery provides better output of the energy, chemicals, and other materials as compared to the conventional methods of refinery. Biomass and waste materials are utilized as raw materials by a series of sustainable technologies to produce valuable products with high economy (Gude and Martinez-Guerra, 2017). The paradigm of biorefinery defines the great hub of scientists from various field of science, such as biochemistry, biology, economics, environmental sciences, and chemical engineering to invent a bio-based framework to utilize renewable resources. Generally, food crops are consumed as basic material for the production of biofuels and other materials which point out various deficiencies and issues for their utilization in a bioeconomy (Cherubini, 2010; Luque et al., 2008). Introduction of a well-managed and integrated approach is in demand which utilizes by-products, waste, and other residues as raw materials to achieve the goal of high production ratio to the feedstock.

At present, waste is a major issue of concern at global level, especially in the developing countries. There could be different types of waste based on origin, such as industrial, agricultural, and solid waste. The food-processing companies are responsible for 50% production of the total waste produced in countries which could be considered as preconsumer type of waste having 60% of the organic matter. According to McKinsey Global Institute report, food waste holds third rank among the 15 recognized resources for the production of economically beneficial products (Dobbs et al., 2011). However, in many cases the food wastes are used for landfilling, composting, animal feed, or as organic matter. But the present scenario of our society demands the introduction of advanced processes for the conversion of food waste in high value and commercial products to attain maximum profit

Refining Biomass Residues for Sustainable Energy and Bioproducts. DOI: https://doi.org/10.1016/B978-0-12-818996-2.00017-X

CHAPTER 7

Wastewater treatment and sludge management strategies for environmental sustainability

Manisha Sharma[a], Ankush Yadav[a], Mrinal Kanti Mandal[b], Shailesh Pandey[b], Supriya Pal[c], Hirok Chaudhuri[d], Sandip Chakrabarti[e], and Kashyap Kumar Dubey[a,f]

[a]Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh, India
[b]Department of Chemical Engineering, National Institute of Technology, Durgapur, India
[c]Department of Civil Engineering, National Institute of Technology, Durgapur, India
[d]Department of Physics, National Institute of Technology, Durgapur, India
[e]Amity Institute of Nanotechnology, Amity University, Noida, India
[f]Bioprocess Engineering Laboratory, School of Biotechnology, Jawaharlal Nehru University, New Delhi, India

1. Introduction

Wastewater treatment plants (WWTPs) were set up for the removal of different contaminants present in wastewater before its discharge into nearby water bodies. These WWTPs generate a lot of a residual waste, known as waste sludge, a by-product of the different processes involved (coagulation, filtration, disinfection, etc.). About 100,000 tn/yr of sludge is produced worldwide with estimated average daily production of more than 1000 tons (Babatunde and Zhao, 2007). Studies estimate a total annual production of 240 million tons of sludge in developed countries (i.e., Europe, USA, and China) only from the WWTPs (Pritchard et al., 2010), while specifically in Europe it will reach up to 13 million tons in 2020 (Gendebien et al., 2010). This sludge is of great environmental concern as it is treated as a waste and, in India, it is discharged into the nearby water bodies or nearby open lands (Kamyotra and Bhardwaj, 2011).

As it is generated from the treatment of wastewater, sludge is an active mixture of water (90%–98%), organic matter (50% of total dry weight) (Martinez-Toledo et al., 2012), dead and alive microorganisms both beneficial as well as pathogenic (Viau et al., 2011), and harmful inorganic (heavy metals such as Cd, Hg, Pb, etc.) and organic contaminants (PAHs, pesticides, etc.) (Gonzalez-Martinez et al., 2016; Rodríguez et al., 2015; Saunders et al., 2016). The actual biological, chemical, and physical composition of sludge varies according to the treatment methods used at the WWTPs (Sales et al., 2011). Sludge is mainly used for landfilling and as a fertilizer in agriculture (Gorazda et al., 2017), while a quantity is utilized for thermal processing. The main constraint on sludge use as a resource rather than as waste is the presence of heavy metals and pathogens. This problem

Circular Economy and Sustainability, Volume 2
https://doi.org/10.1016/B978-0-12-821664-4.00027-3

Aptamer-Based Biosensors for Detection of Environmental Pollutants

10

Meenu Goyal, Ankush, Mukesh R. Jangra, Ritu Batra, and Pardeep Kumar

Abstract

Environmental pollutants monitoring is utmost importance for safety of living being of our ecosystem. Though the traditional detection techniques are capable of accurate analysis of environmental pollutants, there is need to develop fast, real-time and cost-efficient techniques for detecting and monitoring the environmental pollutants. Aptamer-based biosensors have shown promising performance in the detection of environmental contamination due to their high sensitivity, specificity and reusability. The aptasensor acts as an analytical device, which uses an aptamer as a ligand molecule. Aptamer is recently emerging as potential sensing elements that can replace other ligands due to its high temperature stability, low-immunogenicity, low-toxicity, high affinity and high specificity. This chapter explains the advances in development of aptasensor and its applications in the detection of various environmental contaminants.

Keywords

Aptamer · Biosensor · Detection · Environmental pollutants

M. Goyal · Ankush
Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India

M. R. Jangra
Department of Biotechnology, Government Post Graduate College, Hisar, Haryana, India

R. Batra
Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, India

P. Kumar (✉)
Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India

G. S. Yadav et al. (eds.), *Aptamers*, https://doi.org/10.1007/978-981-13-8836-1_10

8

Nanomaterial Biosynthesis and Enzyme Immobilization

Methods and Applications

Indu[1], Ankush Yadav[1], Mrinal Kanti Mandal[2], and Kashyap Kumar Dubey[1]

[1] *Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India*
[2] *Department of Chemical Engineering, NIT Durgapur, Durgapur, West Bengal, India*

8.1 Introduction

Nanotechnology comprises nanomaterials that clarify the construction and exploitation of bulk materials in range of nanometer size (1 nm = 10^{-9} m), (John et al. 2010; Rao et al. 2006). It has great importance as an interdisciplinary science due to its potential for wide application in different industries, including cosmetics, clothing, food industry, medical industry, household appliances, and renewable energies. Along with these applications, other untouched areas of advantage for nanomaterials are also available. Nanotechnology becomes popular and fascinating, with a wide range of applications. These include the interaction of nanomedicine with biological molecules and application in green technology for environmentally friendly and renewable energy solutions for capturing, reserving, and transferring energy (Ghoranneviss et al. 2015).

The theme of nanotechnology is miniaturization and the nanometer-scale structures can lead to a science comprising new devices and technologies. Nanotechnology is a promising technology that exploits nanometer-sized materials. Nanofilteration is used for separation of divalent ions (Ankush et al. 2019). Nanomaterials hold great importance because of their unique electrical, optical, magnetic properties and their huge influence in biofuels, electronics, medicines, and other fields. In the growing era of nanotechnology, biocompatible nanomaterials are being used to replace natural materials in living systems. This chapter explains the manufacture and assembly of nanostructures and their applications in engineering and biology (Prashant et al. 2008).

Immobilization defines the cell or enzyme static to a matrix or support. At industrial level, the demand for increasing shelf life and production of enzymes is growing continuously. In the near future, engineered enzymes will be accessible for all industrial and pharmaceutical processes (Dubey et al. 2019). Therefore, immobilization plays an important role in the availability of enzymes for a continuing a chemical reaction. Immobilization provides the base for the enzyme–substrate reaction, which leads to increased reaction

Green Synthesis of Nanomaterials for Bioenergy Applications, First Edition. Edited by Neha Srivastava, Manish Srivastava, P. K. Mishra and Vijai Kumar Gupta.

CHAPTER 15

Bioeconomy of municipal solid waste (MSW) using gas fermentation

Khushboo[1], Ankush[1], Karuna Yadav[1], Mrinal Kanti Mandal[2], Supriya Pal[3], Hirok Chaudhuri[4], Kashyap Kumar Dubey[1]

[1]Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendergarh, India; [2]Department of Chemical Engineering, National Institute of Technology, Durgapur, India; [3]Department of Civil Engineering, National Institute of Technology, Durgapur, India; [4]Laboratory of Environmental Geochemistry, Department of Physics, National Institute of Technology, Durgapur, India

1. Introduction

In today's world the demand of energy is increasing day by day and till 2035, it will increase up to 40%. Till that time line, the world population is also expected to touch the mark of a billion [1]. The strict rules and regulations concerned with waste and increasing demand of energy are driving the production industry toward greater tenability for better cost and fulfilling the consumer's demand [2]. In that situation, the major problem is not only to fulfill the energy demand but also to decrease emission of fossil carbon for the protection of the environment.

The debris released in the environment because of routine activities from the residential and business area leads to huge production of municipal solid waste (MSW). The basis of MSW relies upon a number of factors like source, area, monetary and cultural status, and lifestyle of the community [3]. The traditional waste dumping approaches result in several environment issues like water pollution and greenhouse gas (GHG) emission. Rather than disposal of the MSW, current research should be concentrated on its usage as energy source [2]. At present, gas fermentation is the most reliable technology for carbon fuel synthesis by industrial waste gases and syngas brought up gasification of MSW [1]. Acetogenic bacteria are the key players of gas fermentation to ferment carbon-rich gases. This technology is valuable over the other entrenched technologies and is assessing the monetary scale for the execution of low carbon fuels and other chemicals [4]. Gasification–fermentation involves a two-step conversion process that leads to the production of fuels and chemicals primarily from crops, wood, and MSW. Via gasification

Current Developments in Biotechnology and Bioengineering. https://doi.org/10.1016/B978-0-444-64321-6.00015-X

CHAPTER

12

Enzyme Engineering

Kashyap Kumar Dubey[*], *Avijit Pramanik*[†], *Ankush*[*], *Khushboo*[*], *Jyoti Yadav*[†]

[*]Bioprocess Engineering Laboratory, Department of Biotechnology, Central University of Haryana, Mahendragarh, Haryana, India [†]Department of Microbiology, Central University of Haryana, Mahendragarh, India

12.1 INTRODUCTION

Any macromolecule that possesses catalytic activity, that is, that enhances any reaction toward forward direction, can be called an enzyme. In nature, most enzymatic activities are carried out by proteins. Types of reactions carried out by any proteinaceous enzymes are very specific, and each enzyme catalyzes specific types of chemical reactions on specific, or very closely related, substrates. Enzymes obtained from various biological sources have huge potential to accelerate a variety of reactions with important applications in many industries. For example, a vast number of enzymes have been commercially used, such as lipase (the biofuel and pharmaceutical industries), xylanase (the paper and pulp industry), lipoxygenase (the food industry), monooxygenase-dependent biocatalysis, and α-amylase (the detergent industry) [1–4].

In general, environmentally hazardous organic solvents are used for the formation of many valuable chemical products. So, there is a tremendous demand for potent alternative biocatalysts that are eco-friendly, yet able to carry out the needed high-value chemical synthesis. The native forms of enzymes are optimally active under the physiological conditions of the organism in which they reside. To be useful under industrial chemical processes, the ideal enzyme should possess the ability to withstand several harsh conditions, such as high temperature, high salinity, and a strong acidic or basic environment, and extreme pH. That's why it is necessary to engineer the enzymes by various enzyme engineering and stabilization techniques for the purposes of industrial and research applications. Advancement in biological tools, as well as computational tools, has given a boost to the field of enzyme engineering by manufacturing novel or improved enzymes suitable for different conditions, and suitable

https://doi.org/10.1016/B978-0-444-64114-4.00012-1

Aptasensor-Possible Design and Strategy for Aptamer Based Sensor

9

Jyoti Yadav, Ankush, Khushboo, Mony Thakur, Karuna Yadav, Manisha Sharma, and Kashyap Kumar Dubey

Abstract

Aptamers have procured immense attention as an evident identification element in biosensor design. Presently various electrochemical, optical, colorimetric, fluorescent, luminescent etc. are in trend because of their small size, flexibility to design, high sensitivity, high selectivity, chemical stability, temperature resistibility and cost effectiveness. The aptasensors have been classified in various groups depending on their configuration, confirmation and conductivity and four basic strategies have been used for designing the aptasensors i.e. target induced structure mode, sandwich mode, target induced dissociation mode and competitive replacement mode turning on the specific bidding process leading to signal variation however certain efforts have been made continuously in the design and operation of aptasensors. Nanotechnology, micromachines, quantum dot etc. have paved a new way in improvement of biosensors however in spite of rapid advancement aptasensors are still immature and need further amelioration.

Researchers are on way to take the tools further for significant advancement in the performance of aptasensors because of their unprecedented advantages. This chapter will give an overview of different types of aptasensors with their designing strategies and methods that have been implemented so far.

Keywords

Aptamer designing · Target induced modes · Aptamer types

J. Yadav · M. Thakur
Department of Microbiology, Central University of Haryana, Mahendergarh, India

Ankush · Khushboo · K. Yadav · M. Sharma · K. K. Dubey (✉)
Department of Biotechnology, Central University of Haryana, Mahendergarh, India

G. S. Yadav et al. (eds.), *Aptamers*, https://doi.org/10.1007/978-981-13-8836-1_9

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते
GOVERNMENT OF INDIA

Controller General of Patents,Designs and Trademarks
Department of Industrial Policy and Promotion
Ministry of Commerce and Industry

Application Details

APPLICATION NUMBER	201711044714
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	13/12/2017
APPLICANT NAME	CENTRAL UNIVERSITY OF HARYANA
TITLE OF INVENTION	"A PAPER STRIP BASED TEST FOR DETECTION OF AMINOGLYCOSIDES GROUP OF ANTIBIOTICS IN WATER AND WASTE WATER
FIELD OF INVENTION	CHEMICAL
E-MAIL (As Per Record)	
ADDITIONAL-EMAIL (As Per Record)	registrar@cuh.ac.in
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	14/06/2019

Application Status

APPLICATION STATUS	**Awaiting Request for Examination**

View Documents

➡ Filed ➡ Published ➡ RQ Filed ➡ Under Examination

➡ Disposed

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते
GOVERNMENT OF INDIA

Controller General of Patents,Designs and Trademarks
Department of Industrial Policy and Promotion
Ministry of Commerce and Industry

Application Details

APPLICATION NUMBER	201811026150
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	13/07/2018
APPLICANT NAME	REGISTRAR, CENTRAL UNIVERSIYT OF HARYANA, MAHENDERGARH
TITLE OF INVENTION	"A RAPID METHOD FOR DETECTION OF AMMONIUM SULPHATE ADULTERATION IN MILK"
FIELD OF INVENTION	FOOD
E-MAIL (As Per Record)	
ADDITIONAL-EMAIL (As Per Record)	ankushrao540@gmail.com
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	17/01/2020

Application Status

APPLICATION STATUS	**Awaiting Request for Examination**

View Documents

➡ Filed ➡ Published ➡ RQ Filed ➡ Under Examination

➡ Disposed

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते
GOVERNMENT OF INDIA

Controller General of Patents,Designs and Trademarks
Department of Industrial Policy and Promotion
Ministry of Commerce and Industry

Application Details

APPLICATION NUMBER	201911027350
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	09/07/2019
APPLICANT NAME	REGISTRAR, CENTRAL UNIVERSITY OF HARYANA, MAHENDERGARH JANT-PALI,-123031
TITLE OF INVENTION	"A RAPID METHOD FOR DETECTION OF UREA IN MILK AND POTABLE WATER"
FIELD OF INVENTION	FOOD
E-MAIL (As Per Record)	
ADDITIONAL-EMAIL (As Per Record)	registrar@cuh.ac.in
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	15/01/2021

Application Status

APPLICATION STATUS	**Awaiting Request for Examination**

View Documents

➡ Filed ➡ Published ➡ RQ Filed ➡ Under Examination

➡ Disposed

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते
GOVERNMENT OF INDIA

Controller General of Patents,Designs and Trademarks
Department of Industrial Policy and Promotion
Ministry of Commerce and Industry

Application Details

APPLICATION NUMBER	201911027351
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	09/07/2019
APPLICANT NAME	REGISTRAR, CENTRAL UNIVERSITY OF HARYANA, MAHENDERGARH JANT-PALI,-123031
TITLE OF INVENTION	"A STRIP BASED METHOD FOR DETECTION OF UREA IN MILK"
FIELD OF INVENTION	BIOTECHNOLOGY
E-MAIL (As Per Record)	
ADDITIONAL-EMAIL (As Per Record)	registrar@cuh.ac.in
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	15/01/2021

Application Status

APPLICATION STATUS	**Awaiting Request for Examination**

View Documents

➡ Filed ➡ Published ➡ RQ Filed ➡ Under Examination

➡ Disposed

International Conference on

New Horizons in Biotechnology

(NHBT 2019)

November 20-24, 2019, Thiruvananthapuram, Kerala, India

CERTIFICATE

This is to certify that **Ankush, Central University of Haryana, Mahendergarh, India** has participated and presented a paper/poster entitled **"Genotoxic activity detection of cytostatic compounds cyclophosphamide, etoposide and paclitaxel in hospital wastewater"** in the International Conference on New Horizons in Biotechnology (NHBT- 2019) jointly organized by CSIR-National Institute for Interdisciplinary Science and Technology, and the Biotech Research Society, India, at Thiruvananthapuram, India, during November 20-24, 2019

Dr Rajeev K Sukumaran
Co-convener

Dr Binod P
Co-convener

Dr K. Madhavan Nampoothiri
Convener

Sr. No. 106

60th Annual Conference of Association of Microbiologists of India (AMI-2019)

&

International Symposium on Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health

Organized by

Central University of Haryana, Mahendergarh -123031, Haryana (India)

15th - 18th November 2019

Certificate

This is to certify that Prof./Dr./Mr./Ms./Mrs. **Mr. Ankush yadav**

from **Central University of Haryana, Mahendergarh**

has Participated / Presented a Poster / Oral Paper entitled *Cytostatic drugs in aquatic environment and their detection by RP-HPLC methods.*

in **AMI-2019.**

Prof. Satish Kumar
Chairman, AMI-2019

Dr. Surender Singh
Dr. Jitendra K. Saini
Organizing Secretaries, AMI-2019

Certificate

TEQIP Sponsored Workshop
on
Recent Trends on Remediation of Contaminated Water Bodies and Soil

BRICS
South Africa
China
Brazil
India
Russia

Certificate of participation

This is to certify that Mr./ Ms./Mrs./Dr. ANKUSH

of CENTRAL UNIVERSITY OF HARYANA

has participated in the TEQIP Sponsored Workshop on **Recent Trends on Remediation of Contaminated Water Bodies and Soil**, organised by Centre for Research on Environment and Water (CREW), NIT Durgapur, held at National Institute of Technology Durgapur, West Bengal, India during March 25 to March 29, 2019. The participant has actively participated in the workshop.

Dr. Supriya Pal	Dr. Hirok Chaudhuri	Dr. Mrinal Kanti Mandal	Dr. Raj Narayan Saha	Dr. Kartik Chandra Ghanta
Workshop Co-ordinator, NIT Durgapur	BRICS SPOC, NIT Durgapur	Workshop Joint Co-ordinator, NIT Durgapur	Co-ordinator, CREW, NIT Durgapur	Chairman, CEC, NIT Durgapur

International Conference on

Bio-Innovation for Environmental and Health Sustainable Developments (BEHSD-2018)

Certificate

This is to certify that .....Mr. Ankush..... has participated/presented a Poster/delevered a lecture in the international conference on Bio-Innovation for Environmental and Health Sustainable Developments (**BEHSD-2018**), jointly organized by the Biotech Research Society, India and CSIR-Indian Institute of Toxicology Research, Lucknow on November 27-28, 2018.

Professor N. Manickam	Professor T.P. Singh	Professor Ashok Pandey	Professor Alok Dhawan
Convener, BEHSD-2018	President, BRSI	General Chair	Director, CSIR-IITR

CSIR-Indian Institute of Toxicology Research
Vishvigyan Bhawan, M.G. Marg, Lucknow

27-11-2018

THE 6th IWA-REGIONAL MEMBRANE TECHNOLOGY CONFERENCE, IWA-RMTC 2018

CERTIFICATE

This is to certify that

Prof./Dr./Mr./Ms. ANKUSH

had Plenary Talk/Invited Talk/Oral/Posters Presentation/Attended the

International Conference "IWA-RMTC 2018"

held from 10-12 December, 2018

Shri. S. Mayor
Co-Chair, IWA-RMTC 2018

Prof. C. N. Murthy
Chair, IWA-RMTC 2018

NATIONAL CONFERENCE
on
BIOTECHNOLOGY: EXPLORING THROUGH INNOVATIONS

BETI 2017

November 13-14, 2017

Certificate of Participation

Prof/Dr./Mr./Ms. ANKUSH *From* Department of Biotechnology, CUH

participated in the two-day National Conference on "BIOTECHNOLOGY: EXPLORING THROUGH INNOVATIONS-2017" *held during November 13-14, 2017 at Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana. He/She presented a paper (~~Oral~~/Poster) Titled* Barapullah drain (New Delhi) water treatment with Chlorella Vulgaris

Convener

हरियाणा केंद्रीय विश्वविद्यालय

Central University of Haryana

(संसद के अधिनियम सं. 25(2009) के तहत स्थापित)

(Established vide Act no. 25 (2009) of Parliament)

जांट-पाली, महेंद्रगढ़(हरियाणा)-१२३०३१

Jant-Pali, Mahendergarh (Haryana)-123031

No./CUH/2021/LIB./1726

Date: 27/12/2021

To

The Head

Department of Biotechnology

Central University of Haryana

Mahendergarh-123031 (Haryana)

Plagiarism Analysis Report

1. Please find enclosed herewith plagiarism analysis report as given below for your reference & further necessary action.

(a) Name of the Researcher : **Ankush**

Name of the Course : Ph.D

Name of the Department : Biotechnology

Class Roll No : 11115

Name of the Supervisor : Dr. Bijender Singh (Professor)

Name of the Co-Supervisor: Dr. Kashyap Kumar Dubey (Associate Professor, JNU) & Dr. Mrinal Kanti Mandal (Associate Professor, NIT Durgapur)

Title of the Thesis : **Study of biodegradation of antineoplastic compounds using white rot fungi**

(b) Reference

S.N.	Software	Submission ID	Date	Word Count	Similarity Index
1.	Turnitin	1735892656	27/12/21	37113	08%

2. A report generated by http://turnitin.com is attached herewith.

27/12/2021

University Librarian

University Librarian
Central Library
Central University of Haryana
Mahendergarh - 123031 (Hr.)

Copy to:-

(i) Controller of Examinations, CUH

(ii) Dr. Bijender Singh, Professor & Head, Department of Biotechnology, CUH

(iii) Dr. Kashyap Kumar Dubey, Associate Professor, School of Biotechnology, JNU, New Delhi

(iv) Dr. Mrinal Kanti Mandal, Associate Professor, NIT Durgapur, West Bengal