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).

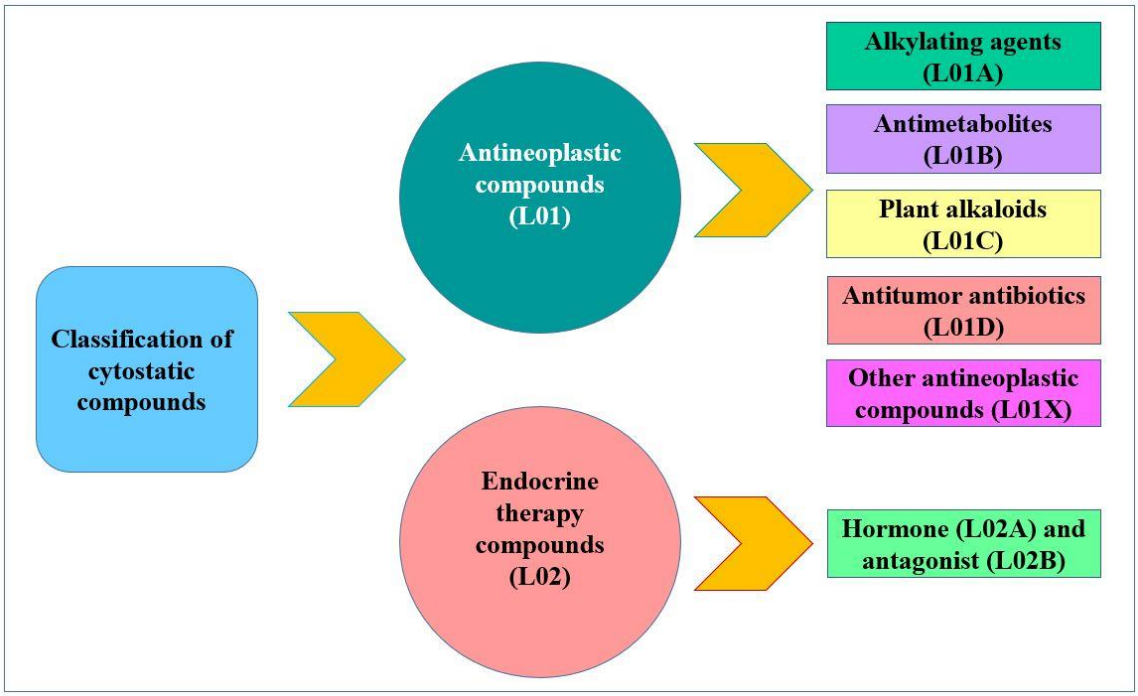

Fig. 2.1. Classification of antineoplastic agents

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.

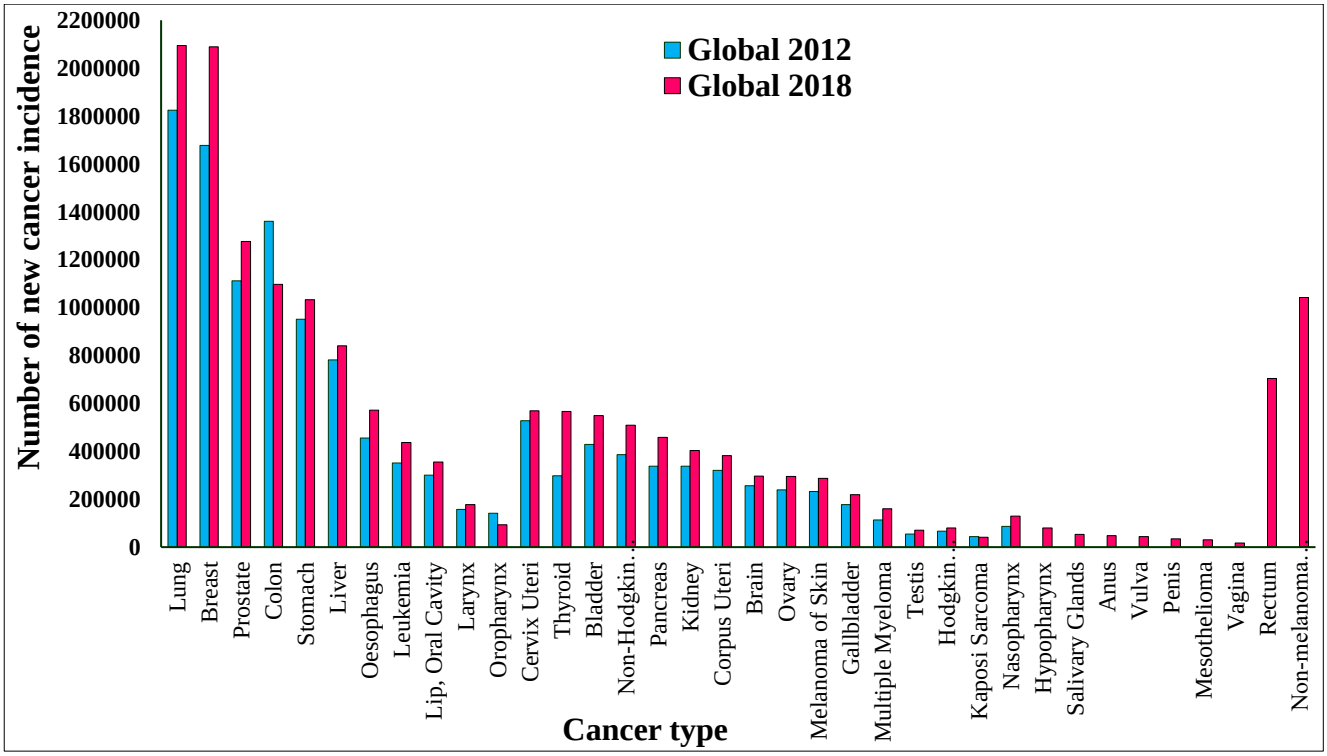

Fig. 2.2. Comparison of worldwide new cancer incidence of year 2012 and 2018

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.

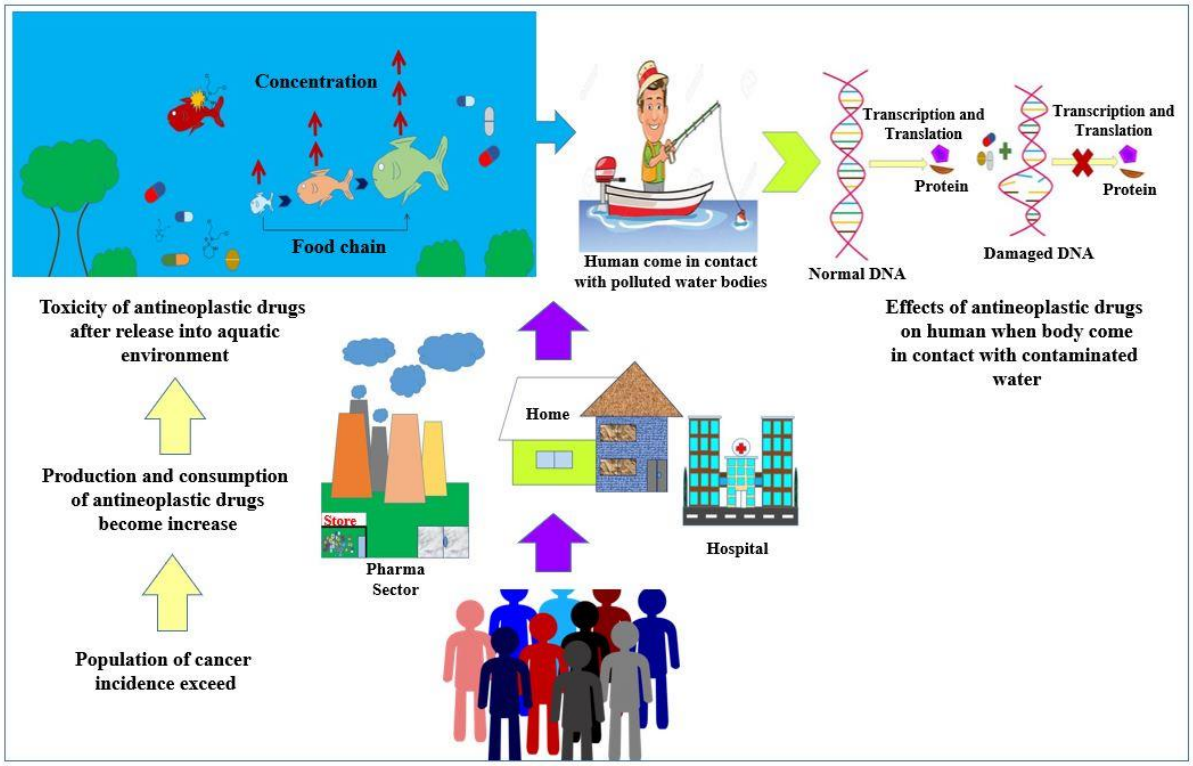

Fig. 2.3. Possible life cycle and negative effect of antineoplastic compounds after release into environment

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	