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Bioaccumulation and toxicity of oxaliplatin in fresh water: A study with *Lemna minor*

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ABSTRACT

Oxaliplatin is the newest platinum-based drug to be used widely in chemotherapy. However, very little is known about its behaviour and toxicity once discharged to the environment. In this study, the freshwater macrophyte, *Lemna minor*, has been exposed to three concentrations of the drug (low, medium and high; 0.4, 10 and 100 μg mL^{-1}, respectively) for seven days according to OECD guidelines and the growth rate, biochemical changes and extent of membrane damage determined. The stability of oxaliplatin was also monitored during the exposures and the accumulation, as both adsorbed and internalised fractions, evaluated by chemical extraction and digestion at the end of the experiment. Oxaliplatin appeared to remain intact throughout, with no measurable metabolite formation in the growth medium, and bioconcentration factors were < 100 mL g^{-1}. The majority of oxaliplatin taken up by *L. minor* (about 66 to 84%) was internalised, presumably through passive diffusion and, possibly, uptake through transporters. Among the end-points tested, only relative growth rate of frond area and ion leakage were adversely affected at the medium and/or high concentrations employed. These observations suggest that oxaliplatin is unlikely to be phytotoxic at concentrations typically encountered in the environment and that *L. minor* is not a suitable biomonitor or phytoremediator of contaminated waters.

1. Introduction

Over the past two decades, the presence and impacts of pharmaceuticals in the environment have gained increasing attention (Besse and Garric, 2008; Laurenson et al., 2014; Ojemaye and Petrik, 2019). Human and veterinary pharmaceuticals and their metabolites enter the aquatic environment largely through treated and untreated hospital and municipal waste waters (Souza and Feris, 2016; Ting and Praveena, 2017), although effluents from manufacturing plants may also act as localised sources (Cardoso et al., 2014). Despite resulting concentrations that are low compared with many other contaminants like metals, nutrients, hydrocarbons and microplastics, the functions and mechanisms of action of pharmaceuticals raise concerns about their chronic effects on non-target organisms (Fent et al., 2006; Walker and McEldowney, 2013).

Cytostatic drugs are a group of antineoplastic agents that are widely used to treat cancer by inhibiting the replication of DNA and intervening in cell division. Their mode of action also ensures that they are toxic to non-cancerous cells, however, suggesting that all eukaryotic organisms could be at risk from environmental exposure (Johnson et al., 2008). Platinum-based coordination complexes are an important and commonly employed group of alkylating cytostatic drugs that may be used either as a single therapeutic modality or in combination with other agents or with radiotherapy (Wang and Guo, 2013). The principal chemicals in this group are cisplatin (*cis*-dichlorodiamineplatinum(II)), carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) and oxaliplatin ((*cis*-[1*R*,2*R*]-1,2-cyclohexanediamine-*N,N′*)oxalato(2-)-*O,O′*platinum(II)). Oxaliplatin, whose structure is shown in Fig. 1, is the newest platinum-based derivative to be internationally approved and used in standard chemotherapy and was developed as a less toxic and more effective alternative to cisplatin. Oxaliplatin is used to treat various solid tumours but most commonly colorectal cancer and is usually administered with fluorouracil and leucovorin as synergists (Kwok et al., 2017). The molecule is characterised by an oxalate (ox) leaving group and a diaminocyclohexane (dach) carrier ligand, and may be thus abbreviated as: [Pt(dach)(ox)] (Alcindor and Beauger, 2011). While the compound itself is highly soluble and appears to be pharmacologically inactive, in plasma the oxalate group is displaced and reactive dichloro (dach) complexes, $[Pt(dach)Cl_2]$, enter the cell as cytotoxins (Jerremalm et al., 2009).

Despite the common and increasing usage of platinum-based cytostatic drugs, very little information exists on their environmental behaviour and toxicity, and with most of the focus to date on cisplatin

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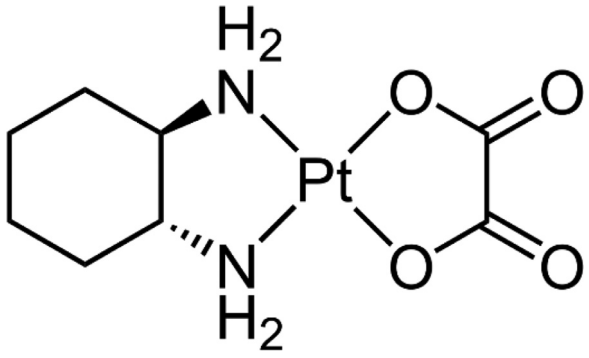

Fig. 1. The structure of oxaliplatin ((*cis*-[1*R*,2*R*]-1,2-cyclohexanediamine-*N*,*N*′)oxalato(2-)-*O*,*O*′platinum(II)).

(Supalkova et al., 2008; Curtis et al., 2010; Easton et al., 2011; Brezovšek et al., 2014; Fonseca et al., 2017). For oxaliplatin, the adsorption to sediment and soil has been investigated under controlled conditions (Turner and Mascorda, 2015; Goykhman et al., 2018) but information on its interaction with and toxicity to biota appears to be limited to a single recent study using *Chlorella vulgaris* (Dehghanpour et al., 2020). Here, it was found to be the least toxic among the three main platinum-based cytotoxic drugs to the microalga according to various end-points.

In the present study, we examine the uptake of oxaliplatin by and its effects on the duckweed, *Lemna minor*, a limnic vascular plant. *L. minor* is commonly encountered in temperate freshwaters and is an important food source and habitat for a range of organisms. The macrophyte is easily cultured and handled and is also often employed in ecotoxicological studies as a representative of higher aquatic plants according to OECD and ISO test guidelines (Drost et al., 2007). Specifically, we study the adsorbed-internalised distribution of oxaliplatin in *L. minor* and the effects of the drug on frond growth and on physiological and biochemical changes to fronds. In addition to providing valuable information on uptake and toxicity, the study also allows us to assess the potential for *L. minor* to act as a biomonitor or phytoremediater in contaminated waters.

2. Materials and methods

2.1. Materials

All plastic-ware and glassware used for culturing and in the experiments were soaked in 0.5% general purpose sodium hypochlorite solution, an antineoplastic inactivator and decontaminant (Benvenuto et al., 1993), and subsequently rinsed with Millipore Milli-Q water (MQW). Work involving the preparation and handling of oxaliplatin was performed on a Chemoprotect cytostatic mat in a Bigneat biological class II safety cabinet while wearing appropriate protective gear.

2.2. Cultivation of Lemna minor

A known and established *Lemna minor* strain (UTCC# 490) was used throughout this study. Stock material was cultured and maintained according to OECD 221, *Lemna sp*. growth inhibition test (OECD, 2006), in transparent plastic tanks (35 cm × 70 cm) filled with Swedish Institute standard growth medium (SIS) in a greenhouse. When required for experimentation, plants were transferred to the laboratory and washed with tap water several times to remove any adhering debris before being surface-sterilised by soaking in 0.5% v/v ethanol (Fisher Scientific HPLC grade) for 30 s, immersing in a solution of 0.5% v/v NaOCl (Fisher Scientific HPLC grade) for 3 min and rinsing with MQW for 15 min (Wang et al. 2012). Plants were then allowed to multiply and acclimate for four weeks in sterilised 100 mm x 20 mm polystyrene petri dishes containing 80 mL of sterile (autoclaved) SIS medium at pH 6.5±0.5 in a Sanyo controlled environment chamber. Temperature was set at 24°C and fluorescent light (OSRAM TL- Tube D36W/Brite Gro 2048, Germany) of intensity 35 μmol m^{-2} s^{-1} was employed for a photoperiod of 14 h light per day. In order to minimise contamination, plants were subcultured once per week (OECD 2006). During this process, phosphate-buffered saline (NaCl) solution (ACS reagents, Sigma-Aldrich) at pH 6.4 was used as an isotonic and non-toxic rinse.

2.3. Experimental procedure

Experiments were performed in triplicate and under the conditions described above for a period of 7 d in 6-well plates with covers containing 10 mL of oxaliplatin-amended SIS and about 100 mg of biomass per well, or in loosely-lidded 100 mL clear polyethylene beakers containing 60 mL of oxaliplatin-amended SIS and about 500 mg biomass per vessel. The concentrations of oxaliplatin, prepared from a stock infusion solution of 5 mg mL^{-1} (Accord Healthcare Ltd, batch PP02066) and dilutions thereof in SIS medium, were 0 (control), 0.4 μg mL^{-1} (1 μM; low), 10 μg mL^{-1} (25 μM; medium) and 100 μg mL^{-1} (250 μM; high).

After the acclimation period, a weighed quantity (with the precise amount dependent on the end-point investigated) of light-green, healthy biomass was transferred to each reaction vessel with the aid of a disposable plastic inoculation loop.

2.3.1. End-points (growth analyses)

To determine relative growth rate based on mass (RGR_{FW}; g g^{-1} d^{-1}), fresh biomass (FW; g) was determined on an analytical balance at the beginning, t_0, and end, t_e, of each 7-d exposure after plants had been blotted with absorbent paper:

$$RGR_{FW} = \left(\ln FW_e - \ln FW_0\right)/\left(t_e - t_0\right) \quad (1)$$

Total frond area (TFA; mm^2) was measured at the beginning and end of each exposure using a scanning plant meter (Delta-T devices, Cambridge) and relative growth rate based on total frond surface area (RGR_{TFA}; mm^2 mm^{-2} d^{-1}) was calculated as follows:

$$RGR_{TFA} = \left(\ln TFA_e - \ln TFA_0\right)/\left(t_e - t_0\right) \quad (2)$$

2.3.2. End-points (physiological analyses)

The percentage leakage of cellular ions as a measure of membrane damage was determined following the protocol of Devi and Prasad (1998) with some modifications. Thus, at the termination of the experiment, approximately 30 mg of fronds from each replicate were immersed in 10 mL of MQW in 15 mL plastic centrifuge tubes. The initial conductivity of the medium, C_1, was measured using a temperature compensated conductivity meter (CMD8500/Walden Precision Apparatus Ltd). Tubes were subsequently placed in a Techne Dri DB-2TC heating block at 80°C for 15 min and cooled over ice with the final conductivity, C_2, measured as above. Percentage ion leakage was derived from $C_1/(C_1+C_2)$.

2.3.3. End-points (biochemical analyses)

Lipid peroxidation is one of the biochemical changes as a symptom of oxidative stress in plants by production of reactive oxygen species (Sharma et al. 2012). The level of lipid peroxidation products can be expressed as 2-thiobartbituric acid (TBA) and was determined after the exposure period by estimation of the relative malondialdehyde (MDA) content following Heath and Packer (1968) with slight modification.

Biomass arising from the exposures which had been stored frozen in a New Brunswick Scientific Ultra low temperature freezer at -80°C was homogenized vigorously in a ceramic pestle and mortar with 10% (w/v) trichloroacetic acid (TCA; Fisher Scientific HPLC grade) in a ratio of 100 mg of tissue to 1 mL of TCA. Material was then kept on ice before centrifuging at 10,000 *g* for 5 min at 4°C in 2.5 ml plastic centrifuge tubes. Supernatants were treated with an equal volume of 0.25% (w/v)

TBA and incubated in a heating block at 95°C for 30 min after tube lids had been pierced with a needle before the contents were rapidly cooled on ice. The absorbance of the TBA-MDA complexes in the supernatants was read at 532 nm and corrected for non-specific turbidity by subtracting absorbance at 600 nm using a UNICAM Spectrophotometer. MDA concentration was determined on a fresh weight basis using 155 mM^{-1} cm^{-1} as an extinction coefficient.

2.3.4. Qualitative observations

Changes in plant appearance, including chlorosis, necrosis, frond size, frond disconnection and gibbosity, were checked and recorded at the beginning and the end of the exposures.

2.4. Oxaliplatin measurements

The stability of oxaliplatin under the exposure conditions was evaluated for a period of 7 d in a series of separate experiments in which 0.4, 10 or 100 $\mu g\ mL^{-1}$ of the chemical was added to HPLC-grade water and to SIS medium with *L. minor*. Thus, at selected times, triplicate 1 mL aliquots were pipetted into 2 mL HPLC vials, and 20 μL were injected into the HPLC column (Water Spherisorb 4.6 mm x 250 mm stainless steel packed with 5.0 μm bonded phase cyano CNRP; Waters Ltd). The column was operated at a flow rate of 1.2 mL min^{-1}, a run time of 10 min and a temperature of 35°C, and separation was achieved under isocratic conditions with a mobile phase of 5 mM phosphate buffer and 1% propan-2-ol (Fisher Scientific HPLC grade) at pH 6.5. A UV diode-array detector at 200-210 nm was employed and Jasco ChromPass version 1.8.6.1 was used to analyse chromatographic data.

The HPLC assay described was validated over the duration of the stability study with acceptance criteria adopted from British Pharmacopoeia and European Pharmacopoeia. Thus, intra-day precision and inter-day precision were validated by the analysis of six, freshly prepared 20 $\mu g\ mL^{-1}$ solutions of oxaliplatin in HPLC-grade water on a daily basis (and over a period of 7 d) with acceptance criteria of < 2% (as relative standard deviation). Linearity was validated from a plot of mean peak height arising from three replicate injections for oxaliplatin concentrations in the range 1.0 to 90 $\mu g\ mL^{-1}$ and a least-squares fitted regression coefficient of > 0.99.

2.5. Platinum concentrations

At the end of an independent series of exposures, concentrations of platinum (Pt) were determined in the growth medium, in *L. minor*, and adsorbed to the container. Thus, 1 mL aliquots of the growth medium were pipetted into screw-capped 15 ml plastic tubes and acidified with 4 mL of 2% HNO_3 (Fisher Scientific Trace Metal Grade). Solutions were analysed for Pt by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Thermo Scientific ICAP 7400). The instrument was calibrated with standards prepared from a 10,000 mg L^{-1} solution that had been diluted in acidified SIS medium and was operated under conditions described elsewhere (Bull et al., 2017). Platinum was analysed at 214.423 nm and 217.467 nm, with both wavelengths returning very similar results and indicating that spectral interferences were unlikely. Emptied vessels were subsequently washed with SIS medium before being rinsed with 50 mL of 0.1 M HNO_3 for 15 min. Rinses were analysed for ^{195}Pt by inductively coupled plasma-mass spectroscopy (ICP-MS; ThermoScientific iCAP RQ ICP-MS) under operating conditions described elsewhere (James and Turner, 2020).

Portions of about 250 mg of fresh biomass were washed three times in MQW and blotted dry before being frozen at -80 °C and freeze-dried in an Edwards Super Modulyo freeze-drier. Dried and re-weighed samples were then added to 10 mL of 3 mM of ethylene diamine tetra-acetic acid (EDTA; ACS reagent, Sigma-Aldrich) for approximately 20-25 min in a series of 15 mL polypropylene centrifuge tubes to release adsorbed (extracellular) metal (Hassler et al., 2004). Meanwhile, separate 250 mg portions biomass were dried overnight in a Gollenkamp incubator at 72°C before being re-weighed and completely digested in 10 mL of 16 M HNO_3 in 60 mL centrifuge tubes in a water bath at 85°C for 48 h. MQW-diluted digests and EDTA extracts were analysed by ICP-MS as above.

Aqueous and biomass concentrations of OP in the exposures are presented in terms of Pt after correction for any metal returned in the corresponding controls, and concentrations of Pt accumulated by *L. minor* are given on a dry weight basis after correction for the water content of biomass. Intracellular Pt concentrations were derived by subtracting extracellular (EDTA-extractable) concentrations from total (acid digestible) concentrations.

Table 1
Concentrations (square brackets) and distributions (as K_D and BCF) of Pt arising from 7-d exposure of different quantities of oxaliplatin to *L. minor*. Abbreviations are explained in the accompanying text and errors represent the standard deviation about the mean of three independent experimental measurements.

exposure	low	medium	high
[OP]-added, $\mu g\ mL^{-1}$	0.4	10	100
[Pt]-added, $\mu g\ mL^{-1}$	0.2	4.91	49.1
$[Pt]_{aq}$, $\mu g\ mL^{-1}$	0.20±0.07	4.27±0.16	34. 6±0.52
$[Pt]_{ext}$, $\mu g\ g^{-1}$	1.60±0.21	32.5±1.65	506.0±74.2
$[Pt]_{tot}$, $\mu g\ g^{-1}$	5.06±1.99	138.2±34.5	3253±645. 1
$[Pt]_{int}$, $\mu g\ g^{-1}$	3.46±1.83	120.1±32.2	2747±572
$[Pt]_{int}/[Pt]_{tot}$, %	66.0±10.2	78.3±3.73	84.3±0.98
K_D, mL g^{-1}	8.74±3.56	7.59±0.77	14.6±2.11
BCF, mL g^{-1}	26. 4±10.3	32.5±8.91	94.0±18.6

3. Results

3.1. Oxaliplatin stability

Fig. 2 shows the mean measured concertation of oxaliplatin, $[OP]_{aq}$, as a function of time after being added at three different concentrations (low, medium and high) to HPLC-grade water and to SIS medium in the presence of *L. minor*. Compared with the original concentration, there is no clear reduction over the time-course for any oxaliplatin concentration employed or in either pure water or the growth medium, and additional peaks in the region of the oxaliplatin peak were never observed on the chromatograms.

3.2. Concentrations and partitioning of Pt

Table 1 shows the concentrations and distributions (partitioning) of Pt, as a measure of oxaliplatin and as determined by ICP, at the end of a series of exposures. Note that adsorption of Pt to the interior surfaces of the polyethylene beakers was < 0.3% of the quantity added in each exposure suggesting that chemical artefacts arising from sample containment are minimal. Thus, [OP]-added is the concentration of oxaliplatin added to each exposure, [Pt]-added represents the equivalent concentration of Pt added at the beginning of the experiment (and where [Pt] = 0.491*[OP]) and $[Pt]_{aq}$ is the concentration of Pt remaining in the aqueous phase after 7 d; $[Pt]_{ext}$ and $[Pt]_{tot}$ are the extracellular and total concentrations of Pt in *L. minor* after 7 d, respectively, determined by EDTA extraction and HNO_3 digestion, respectively, and converted to a dry weight basis, and $[Pt]_{int}$ is the internalised concentration, derived from the difference between $[Pt]_{tot}$ and $[Pt]_{ext}$. Also shown are the percentage of Pt accumulated by *L. minor* that is internalised (= $[Pt]_{int}/[Pt]_{tot}$*100%), the bioconcentration factor, BCF (mL g^{-1}), defining the concentration of Pt in the organism relative to the concentration in the surrounding aqueous medium at the end of the exposure (= $[Pt]_{tot}/[Pt]_{aq}$), and an adsorption constant, K_D (mL g^{-1}), that defines the concentration of Pt adsorbed to the surface of the plant relative to the concentration in the aqueous medium at the end of the exposure (= $[Pt]_{ext}/[Pt]_{aq}$). Results reveal that the majority of Pt accumulated by *L.*

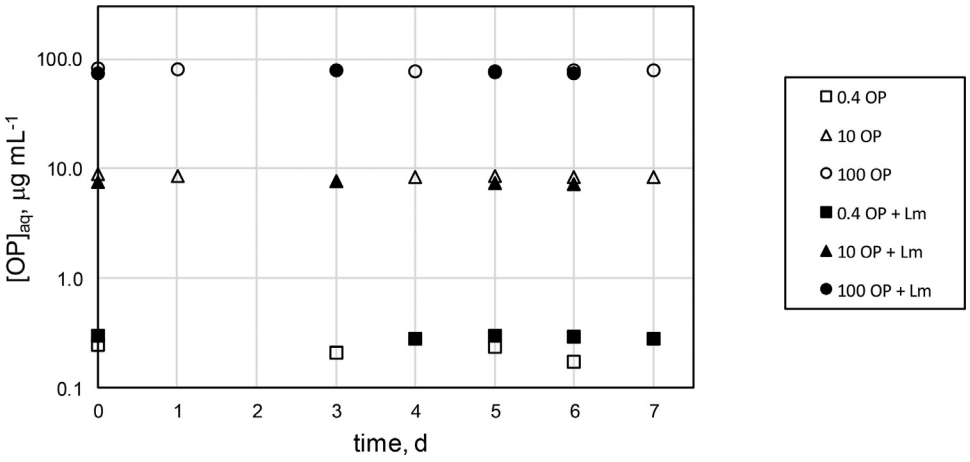

Fig. 2. Mean concentrations of aqueous oxaliplatin, $[OP]_{aq}$, in conditions replicating the exposures: 0.4 OP = 0.4 μg mL^{-1} oxaliplatin in HPLC-grade water; 10 OP = 10 μg mL^{-1} oxaliplatin in HPLC-grade water; 100 OP = 100 μg mL^{-1} oxaliplatin in HPLC-grade water; 0.4 OP + Lm = 0.4 μg mL^{-1} oxaliplatin in SIS medium in the presence of *L. minor*; 10 OP + Lm = 10 μg mL^{-1} oxaliplatin in SIS medium in the presence of *L. minor*; 100 OP + Lm = 100 μg mL^{-1} oxaliplatin in SIS medium in the presence of *L. minor*. Note that errors are not shown but were smaller than the size of the symbols (relative standard deviations < 3%; n = 3).

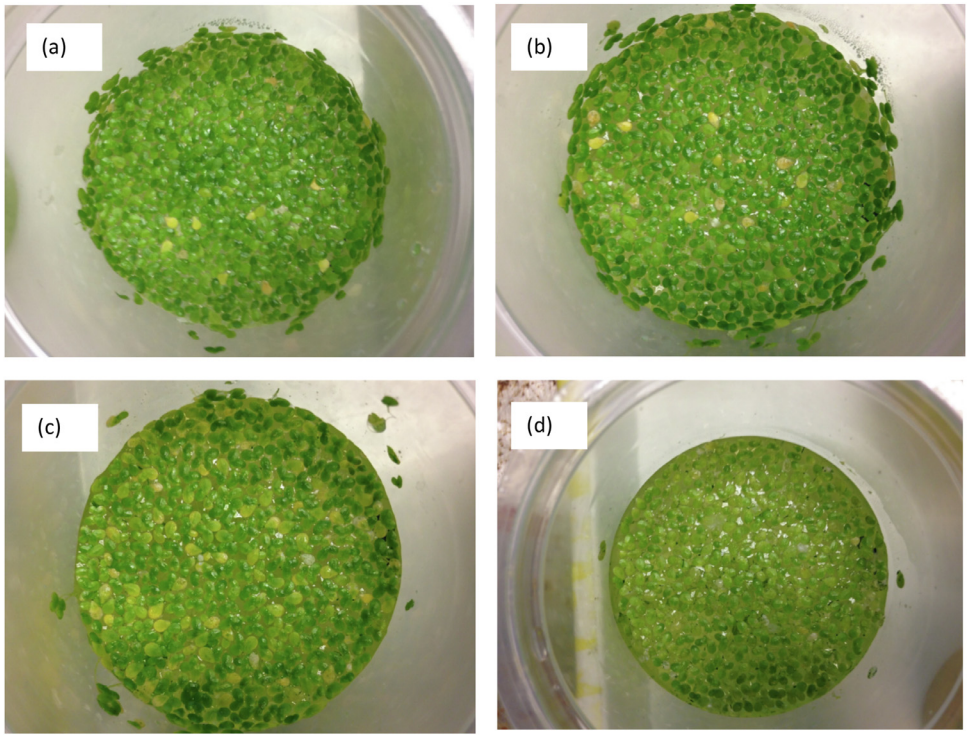

Fig. 3. Visual appearance of *L. minor* in 60 mL beakers and exposed to different concentrations of oxaliplatin: (a) 0 μg mL^{-1}, (b) 0.4 μg mL^{-1}, (c) 10 μg mL^{-1}, (d) 100 μg mL^{-1}.

minor at all added oxaliplatin concentrations is internalised and that the BCF increases with increasing added concentration of the drug.

3.3. Visual and quantitative end-points

In the control exposures, plants were healthy and green, with a few individuals displaying loss of colour (yellow or white). Increasing concentrations of oxaliplatin were accompanied by increasing loss of frond colour or an increasing number of fronds that were discoloured (see Fig. 3), and at the high concentration there was some detachment of fronds from roots.

Quantitative end-points arising from the growth, physiological and biochemical analyses of 7-d exposures are shown in Fig. 4. Indicated in each case are significant differences ($p < 0.05$) from the control according to one-way analysis of variance and after confirmation for normality (Shapiro-Wilk test) and equal variance (Brown-Forsythe test). Thus, there is a significant reduction in growth rate based on frond area at the highest concentration of oxaliplatin employed, and a significant increase in ion leakage at added concentrations of 10 and 100 μg mL^{-1}. However, exposure to oxaliplatin results in no statistical differences in growth rate based on biomass and MDA content as a measure of lipid peroxidation relative to the corresponding controls.

4. Discussion

A critical characteristic of Pt-based drugs in experiments of the nature described here is their propensity to degrade or transform in aqueous solutions, as they are designed to do from a pharmacological perspective. The most abundant nucleophile in the SIS medium is chloride (~ 0.5 mM), with the oxaliplatin concentrations employed in our exposures yielding a [Cl$^-$]:[oxaliplatin] molar ratio of between 2 and 500. Previous studies have reported the formation of $[Pt(dach)(ox)Cl]^-$, $[Pt(dach)Cl_2]$ and $[Pt(dach)Cl(OH_2)]^+$ in aqueous solutions within a few hours at [Cl$^-$]:[oxaliplatin] ratios similar to the maximum values used here (Jerremalm et al., 2009; Mehta et al., 2015). However, these studies were undertaken at significantly higher temperatures (37 to 42°C). No clear difference in chromatographic peak heights between equal oxaliplatin concentrations in pure water, in which Cl$^-$ is absent,

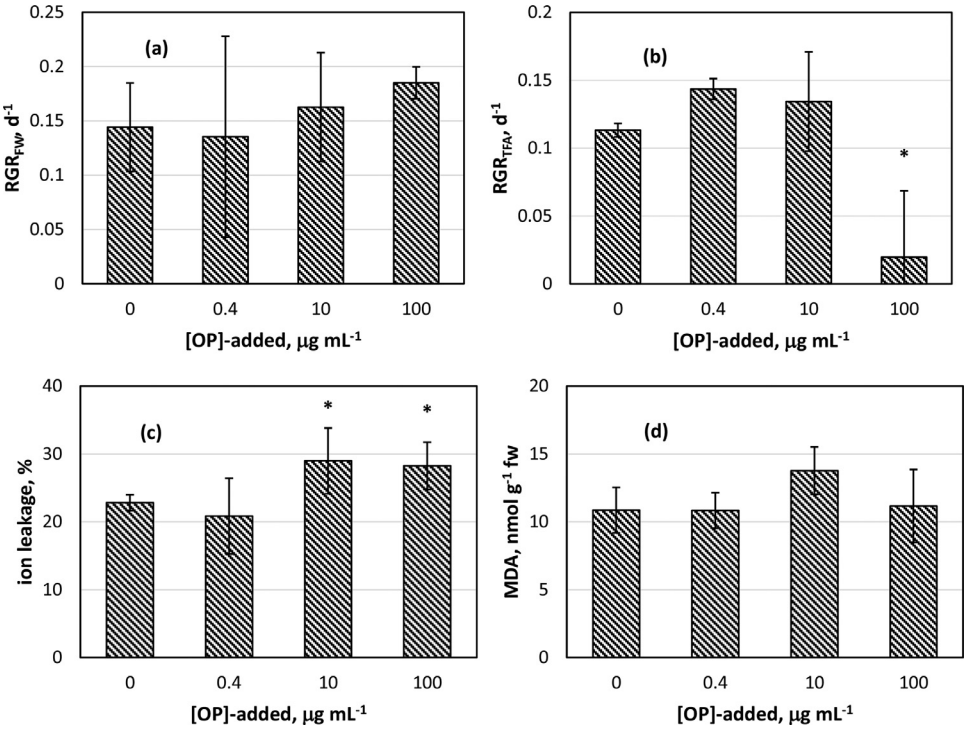

Fig. 4. Relative growth rates (a and b), ion leakage (c) and malondialdehyde content (d) after 7-d exposure to different added concentrations of oxaliplatin ([OP]-added). Errors represent standard deviation about the mean of three independent measurements and asterisks denote a significant difference from the corresponding control ([OP]-added = 0 μg mL^{-1}).

and the SIS medium containing *L. minor*, coupled with no reduction in peak height over the time course of the experiment suggest that oxaliplatin itself, [Pt(dach)(ox)], may be stable under the experimental conditions employed here. However, because some species may not have been detected by the UV detection system in the HPLC assay (Liu, 2016), when we refer to oxaliplatin in the discussion below we acknowledge that small but significant contributions from unidentified chloro-based or aquated transformation products may well have evolved during the exposures.

The BCF defines the bioaccumulation of a contaminant by a plant (and here by the roots and fronds) relative to the amount in ambient water and for oxaliplatin is low compared with other metallic contaminants studied under similar conditions and timeframes. For example, while BCFs for oxaliplatin are < 100 mL g^{-1} (Table 1), heavy metal ions (including oxyanions) typically exhibit values on the order of thousands to tens of thousands of mL g^{-1} for *L. minor* (Weltje et al., 2002; Uysal, 2013; Clement and Lamonica, 2018). The relatively low accumulation and adsorption of oxaliplatin by the duckweed is consistent with the high solubility and hydrophilicity of the molecule (solubility = 7.9 g L^{-1} and log K_{ow} = -0.05; Vyas et al., 2014) and its rather low reactivity towards biochemicals and geosolids (sand, soil and sediment) compared with its charged metabolites like [Pt(dach)(OH$_2$)$_2$]$^{2+}$ and [Pt(dach)Cl(OH$_2$)]$^{+}$ (Verstraete et al., 2001; Alcindor and Beauger, 2011; Turner and Mascorda, 2015; Goykhman et al., 2018). Because of the small size and lack of charge of the oxaliplatin molecule, adsorption is likely to involve non-specific molecular interactions with biomolecules like membrane proteins and lipids at the plant surface and internalisation is predicted to be passive. However, recent empirical evidence suggests that active incorporation of Pt drugs, including oxaliplatin, into cells may also take place through certain transport systems (e.g. copper influx and efflux transporters) (Martinez-Balibrea et al., 2015; Martinho et al., 2019). Despite uncertainties in the mechanisms of interaction, *L. minor* is not predicted to be particularly useful for the biomonitoring or bioremediation of water contaminated by oxaliplatin (e.g. hospital wastewater) unless environmental conditions favour the formation of reactive metabolites.

In addition to being relatively low, BCFs in *L. minor* increase with increasing concentration of oxaliplatin added to the medium (Table 1). Moreover, when the mode of accumulation is considered, it appears that intracellular oxaliplatin rather than extracellular (adsorbed) oxaliplatin is largely responsible for this increase. An increase in accumulation that is proportionally greater for the internal fraction does not appear to be accompanied by a shift in the speciation of oxaliplatin according to experimental results reported above (Fig. 2). Moreover, an increase in concentration of oxaliplatin relative to [Cl^{-}] (a constant in the medium) would be predicted to result in a reduction in the tendency to form more reactive metabolites. We note enhanced ion leakage (but not lipid peroxidation) at the medium and high oxaliplatin concentrations employed and suggest that cell membrane damage allows for greater internalisation of the compound.

Two end-points were affected by oxaliplatin in the exposures but not in a clear dose-dependent manner and only at the high or medium concentrations employed (relative growth rate based on frond area and ion leakage). Oxaliplatin has been shown to be the least toxic platinum-based antineoplastic drug towards the freshwater microalga, *Chlorella vulgaris*, with cisplatin being of greatest environmental concern (Dehghanpour et al., 2020). Regarding *L. minor*, data arising from exposure to cisplatin for 4 d reported by Supalkova et al. (2008) (including a 50% reduction in growth at 1.5 μg mL^{-1}) suggests that this drug is considerably more toxic than oxaliplatin to the duckweed. More generally, platinum-based drugs appear to be less toxic to plants than many other contaminants, and in particular herbicides, presumably because they were specifically developed as alkylating agents for damaging DNA and destroying cancerous human cells. Thus, such drugs appear to exhibit a range of toxicities at low concentrations to various animals, including aquatic fauna (Gajski et al., 2016; Fonseca et al., 2017), but there is no evidence that they inhibit photosynthesis in algal chloroplasts, and certainly at environmentally relevant concentrations (Dehghanpour et al., 2020). These characteristics also suggest that plants may not be effective biomonitors for cytotoxic drugs or act as significant vehicles for food-chain transfer, and certainly at concentrations anticipated in the aquatic environment (on the order of pg L^{-1}; Vyas et al., 2014).

The present study serves as a starting point for understanding both the bioaccumulation and toxicity of a drug that is increasingly used in the treatment of cancer (Wang and Guo, 2013). Further studies should

focus on the toxicity of mixtures of such drugs which are combined in cancer treatment and discharged together into the environment as evidence suggests that mixtures can display synergistic or antagonistic effects (Brezovšek et al., 2014). It is also recommended that studies of oxaliplatin be extended to estuarine conditions where increasing quantities of chloride and bivalent cations are predicted to facilitate the formation of more reactive metabolites of the drug.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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