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Evaluation of Phenolic Compound Toxicity Using a Bioluminescent Assay with the Fungus *Gerronema viridilucens*

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Abstract: Basidiomycetes (phylum Basidiomycota) are filamentous fungi characterized by the exogenous formation of spores on a club-shaped cell called a basidium that are often formed on complex fruiting bodies (mushrooms). Many basidiomycetes serve an important role in recycling lignocellulosic material to higher trophic levels, and some show symbiotic relationships with plants. All known bioluminescent fungi are mushroom-forming basidiomycetes in the order Agaricales. Hence, the disruption of the basidiomycete community can entirely compromise the carbon cycle in nature from fungi to higher trophic levels. The fungus *Gerronema viridilucens* was used in the present study to investigate the toxicity of a phenolic compound series based on the inhibition of its bioluminescence. The median effect concentration (EC50) obtained from curves of bioluminescence inhibition versus log [phenolic compound] showed that 2,4,6-trichlorophenol was the most toxic compound in the series. The log EC50 values of all phenolic compounds were then used for the prediction of their toxicity. The univariate correlation of log EC50 values obtained from 6 different phenolic compounds was stronger with the dissociation constant (pK_a) than with 1-octanol/water partition coefficient (K_{OW}). Nevertheless, the toxicity can be better predicted by using both parameters, suggesting that the phenol-driven uncoupling of fungus mitochondrial adenosine triphosphate synthesis is the origin of phenolic compound toxicity to the test fungus. *Environ Toxicol Chem* 2020;39:1558–1565.© 2020 SETAC

Keywords: Caffeic acid cycle; Luciferase; Mitochondrial impairment; Tropical ecotoxicology

INTRODUCTION

Phenolic compounds are widely used in the pharmaceutical, oil, paint, explosive, polymer, paper, wood, and agrochemical industries. They are defined as priority pollutants and are regulated by environmental protection legislation around the world (Igbinosa et al. 2013; Zhou et al. 2015). Inappropriate industrial or domestic discard of these compounds can damage the environment due to their potential toxicity to microorganisms, plants, and animals (Crawford et al. 2008; Krastanov et al. 2013; Tsai 2013; Duan et al. 2018). These organic compounds are usually persistent and difficult to biodegrade by microorganisms (Ba Bui et al. 2012). Nonetheless, many bacteria and fungi can decompose phenolic substances by extracellular enzymes or metabolic processes (Krastanov et al. 2013), thereby mitigating their toxic action in nature. The investigation of the toxicity of phenolic compounds based on an efficient, very bright model bioluminescent fungus can provide clues to understanding their mechanism of action and to select more suitable fungal species for bioremediation (Gadd 2001; Zhou et al. 2011; Al-Khalid and El-Naas 2012).

Although many basidiomycetous fungi are beneficial for forest ecosystems and agricultural crop development, numerous additional species are phytopathogenic. For instance, 2 bioluminescent basidiomycetes, *Armillaria mellea* and *Mycena citricolor*, are phytopathogens of pine trees and coffee plants, respectively (Ventura et al. 2015). Importantly, ecotoxicological bioassays and prediction models using basidiomycetes can also contribute to the development of fungicides with more effectiveness against phytopathogens and with lower toxicity to nontarget organisms. In addition, the availability of ecotoxicological assays and biological models to

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predict toxicity to a key organism in a particular environmental niche is necessary for the risk assessment of chemical substances (Zhou et al. 2015; Clausen and Trapp 2017).

Unfortunately, bioassays demand time and intensive work to conduct the experiments and to grow the organisms (even though some of these processes can be speeded up). In this context, the prediction of toxicity by univariate or multiparametric linear free-energy relationships is a labor- and time-saving method to anticipate the extent of damage caused by environmentally adverse compounds. A number of ecotoxicological studies were conducted with yeasts and some Ascomycete fungi to predict the toxicity of chlorophenols originating from pulp bleaching in the paper industry, in wood-protecting products, in pesticides, and in combustion processes (Cohen et al. 1988; Briens et al. 1999; Garg et al. 2001; Roy and Sanyal 2006; Çabuk et al. 2012). Although sophisticated multiparametric regressions using dozens of molecular factors can describe the toxicity of a chemical series to a target organism, they can lack interpretability at the molecular level (Zhou et al. 2015).

Toxicity data for white-rot basidiomycetes are very scarce in the literature (Leontievsky et al. 2000; Mendes and Stevani 2010; Mendes et al. 2010). The toxicity of chlorophenols to yeasts disclosed that dissociation constant (pK_a) and 1-octanol/water partition coefficient (K_{OW}) values play an important role in describing their toxicity. Toxicity usually increases with the phenolic compound hydrophobicity at a given pH, which is suggested by the reported correlation with increasing values of either K_{OW} (Arold et al. 1988; Zhao et al. 2009) or the liposome/water partition coefficient (K_{lipw}; Vaes et al. 1997, 1998a, 1998b; Escher and Schwarzenbach 2002). Importantly, no systematic study has been reported to date on the toxicity of a broad range of low-molecular-weight substituted phenolic compounds to basidiomycetes.

In the present study the toxicity of 6 different phenolic compounds (phenol, 2,4,6-trichlorophenol, 4-methoxyphenol, 4-chlorophenol, 4-cyanophenol, and 4-nitrophenol) to *Gerronema viridilucens* was determined using a luminescent bioassay (Mendes and Stevani 2010; Stevani et al. 2013). Univariate and bivariate linear regressions were determined using log median effect concentration (EC50), pK_a and log K_{OW}. The toxicity mechanism of these phenolic compounds to the fungus was then inferred at the molecular and biochemical levels.

MATERIALS AND METHODS

Chemicals and solutions

All phenolic compounds (Sigma-Aldrich; ACS purity >99%) were used as received. Food-grade sugar cane molasses (55.94% sucrose) was graciously donated by Usina São José da Estiva. Stock solutions of phenolic compounds were prepared in sterilized deionized water containing 0.050% (w/v) Triton X-100 (Sigma-Aldrich) at pH 5.5 (adjusted with 100 mM NaOH or 100 mM HCl solutions, both from Merck; ACS). Triton X-100 was added to increase the mycelium wettability, thereby avoiding the formation of surface water droplets (Ventura et al. 2015). Phenolic compound stock solutions were prepared and aliquoted to obtain 0.01 to 20 mM phenol, 0.10 to 20 mM 4-nitrophenol, 0.10 to 20 mM 4-cyanophenol, 0.01 to 76 mM 2,4,6-trichorophenol, 1.0 to 320 mM 4-chlorophenol, and 1.0 to 320 mM 4-methoxyphenol solutions in the cell cultures.

Gerronema viridilucens isolate CCB691 (Instituto Botânico, São Paulo, SP, Brazil) was obtained from mushrooms collected in September 2003 at Parque Estadual Turístico do Alto Ribeira, near the municipality of Iporanga (State of São Paulo, Brazil; Desjardin et al. 2005). The holotype is deposited in the Instituto Botânico herbarium in São Paulo, Brazil (SP307883), and the isotype in the Harry D. Thiers Herbarium at San Francisco State University (San Francisco, CA, USA).

Equipment

Fungal culture media were prepared using deionized water (Milli-Q System; Millipore Simplicity), sterilized in an autoclave (Phoenix, model AB42) at 120 °C for 30 min, and manipulated in a laminar flow hood (Trox, model 2711). *Gerronema viridilucens* cultures were maintained in a climatic chamber (Binder, model KBWF 240) at 25 °C and 80% humidity. Bioluminescence measurements were performed using a microplate luminometer (Tecan, model Infinite M200) with integration time set at 1.5 s/well. To prevent disturbing the culture by shaking the dishes and to improve the accuracy of the bioluminescent assay, a special opaque plastic adapter was designed for the luminometer (Mendes and Stevani 2010; Stevani et al. 2013), allowing the measurement of 6 dishes simultaneously.

Fungal bioluminescent assay

The protocol used in the bioassay was previously described in detail (Mendes et al. 2008; Mendes and Stevani 2010; Stevani et al. 2013). In summary, *G. viridilucens* mycelium was cultivated in 35-mm Petri dishes on agar medium (2.5 mL) under optimized conditions: 1.0% sugar cane molasses, 0.10% yeast extract, at 25 °C, pH 6.0 (unbuffered), and 80% humidity. The assay was performed on the 10th day of inoculation, when the total initial light emission (bioluminescence $[BL]_{initial}$) was determined using a microplate luminometer. Cultures showing similar $BL_{initial}$ intensities (<10% difference in relative light emission) were grouped and considered independent replicates. Aliquots (500 µL) of aqueous solutions of phenolic compounds and controls were applied over the mycelium surface, and the dishes were closed and kept in the dark in a climatic chamber at 25 °C and 80% humidity over 24 h. Afterward, the dishes were opened, and the total final light emission was once again recorded (BL_{final}). The bioluminescence inhibition (BL_{inhib}) was calculated by the ratio of final-to-initial light emission [$BL_{inhib} = 100 - (100 \times BL_{final}/BL_{initial})$]. Each phenolic compound concentration was tested in triplicate.

The set of phenolic compounds was carefully chosen for being relevant to evaluate the cooperative steric and electronic factors potentially involved in their toxicity. Thus, 2,4,6-trichlorophenol was added to the phenolic compound series due to its well-known toxicity and bulkiness arising from 2 chlorines in the *ortho* position.

Quantification of phenolic compounds in agar medium

The measured concentration of phenolic compound applied to the surface of the agar plates was determined by adapting the Prussian Blue method (Budini et al. 1980). Solutions of phenolic compounds were prepared in deionized water containing 0.05% (m/v) Triton X-100 at unbuffered pH 5.5 (diluent). Aliquots (500 µL) of the different phenolic compound concentrations (0.5–20 mM; Supplemental Data, Table S1) were added onto the surface of the optimized culture medium.

The agar medium surface was washed after 24 h of exposure with 4.5 mL of the diluent and reserved. The agar medium was removed from the Petri dish, divided into lower and upper sections (each one containing half of the medium, ~1.5 mL), and melted in a beaker using a heating plate. The heating procedure was conducted under controlled conditions to prevent any volatilization of phenolic compound or degradation. The concentration of phenolic compound was then determined spectrophotometrically (Hitachi, model U-2010) at 700 nm by adding to an assay tube 100 µL 8 mM potassium ferrocyanide (Sigma-Aldrich; ACS grade ≥ 99%), 100 µL 0.1 M ferric chloride (Sigma-Aldrich; ACS grade ≥ 98%) in 0.1 M HCl (Merck; ACS grade 37%), and 25 µL melted media. The final solution volume was adjusted to 2.5 mL with the diluent. The calibration curves were obtained by plotting the absorbance versus phenolic compound concentration. A solution containing 25 µL melted media in a final volume of 2.5 mL diluent was used as a blank. All concentrations were determined in triplicate.

Data analysis

The experimental EC50 values were obtained by fitting the concentration–response curve using a sigmoidal model (Equation 1). The EC50 values are defined as the phenolic compound concentration that causes 50% bioluminescence inhibition compared with the control.

$$BL_{inhib} = \frac{BL^{min} - BL^{max}}{1 + \left(\frac{C}{EC50}\right)^{n}} + BL^{max} \quad (1)$$

where BL_{inhib} is the bioluminescence inhibition, BL^{min} and BL^{max} are the minimum and maximum values of BL_{inhib}, C is the phenolic compound concentration, and n is the Hill number (curve slope; Mendes and Stevani 2010; Stevani et al. 2013).

Standard-deviations were not used as weight in either univariate or multivariate linear regressions obtained by using the Linear and Multivariate Fit models as implemented in Origin 2016 (OriginLab).

RESULTS AND DISCUSSION

Distribution of phenolic compounds in the medium

The measured concentration of phenolic compounds and their distribution in the agar medium was evaluated to verify whether they remained in the surface aqueous layer or diffused into the agar medium layer. This is a crucial issue, because the assay relies on the application of aqueous solutions of the toxicant onto the mycelium, which grows primarily on the surface of the agar medium. If the phenolic compounds were confined only to the surface layer, the mycelium would be exposed to a phenolic compound concentration very similar to the nominal one. Conversely, if the phenolic compound diffused into the agar medium, the concentration to which the mycelium was exposed would be lower than the nominal one and homogeneously spread throughout the volume occupied by the agar medium. Distribution of phenolic compounds in different sections of the agar medium was evaluated. Experiments were not conducted with phenolic compounds bearing strong electron-withdrawing groups like 4-nitrophenol and 4-cyanophenol because they do not respond to the Prussian Blue method. After 24 h, the time required for the toxicological assay, the results obtained with 4-methoxyphenol, 4-chlorophenol, 2,4,6-trichlorophenol, and phenol showed that the phenolic compounds were evenly distributed in the culture medium. Hence, the solution of the phenolic compound (500 µL) was diluted by the agar medium (2.5 mL) by 6-fold (Supplemental Data, Table S1).

Fungal bioluminescent assay and phenolic compounds toxicity

Bioluminescence depends on molecular oxygen to occur and therefore is closely connected to respiratory activity (Barros and Bechara 1998; Baader et al. 2015; Bechara et al. 2017). Hence, the inhibition of bioluminescence (BL_{inhib}) is an early endpoint that signals the aerobic metabolic status of the fungus. The EC50 values were obtained from curves of BL_{inhib} versus log[phenolic compound]; Figure 1 and Supplemental Data, Tables S2 and S3, fitted using Equation 1.

Overall, the phenolic compounds tested caused inhibition of light emission. The results obtained indicate that the phenols bearing electron-withdrawing groups, and therefore weakly acidic, were more toxic than the electron-donating phenol (4-methoxyphenol). Steric hindrance is also important to understand the toxic effects; at both ends of the tested series, steric-hindered 2,4,6-trichlorophenol is approximately 35-fold more toxic than 4-methoxyphenol (Table 1).

Phenolic compounds are toxic for both aquatic and soil organisms (Escher and Schwarzenbach 2002; Miyazaki et al. 2002; Zhou et al. 2015; Clausen and Trapp 2017). However, each ecotoxicological bioassay protocol is specific for the tested organism, with distinct EC50 units, observed endpoints, and exposure times. Therefore, direct comparison of EC50 values can lead to erroneous conclusions. In this context, the use of correlations with known parameters to predict the toxicity to an organism is more relevant and reliable for comparing diverse species (Miyazaki et al. 2002). For instance, log K_{OW} was used to predict the toxicity to very different organisms such as the earthworm *Eisenia fetida*, the crustacean *Daphna magna*, the fish *Oryzias latipes*, and the algae *Selenastrum capricornutum*

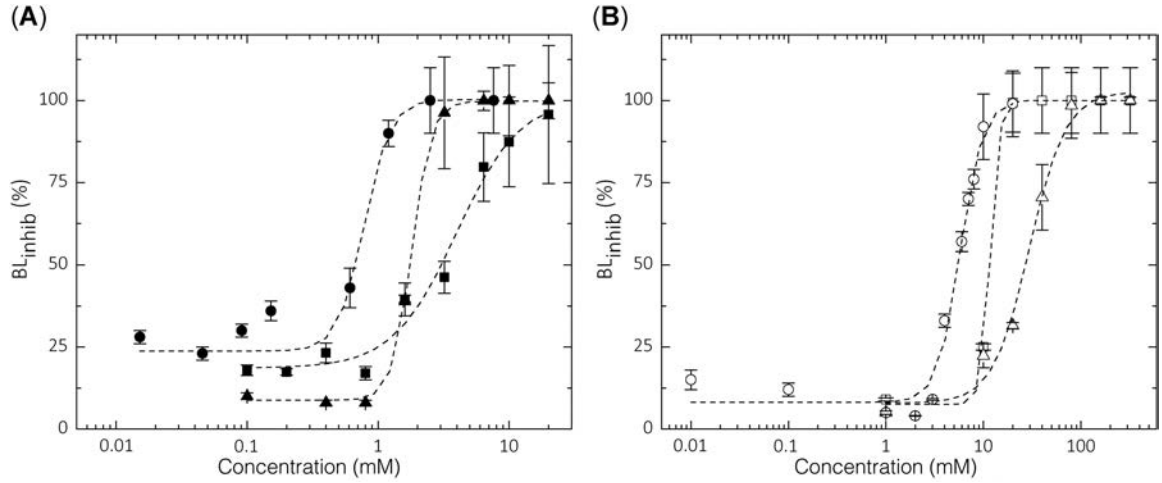

FIGURE 1: Dependence of the bioluminescence inhibition (BL_{inhib}) values obtained from the bioluminescent assay with the fungus *Gerronema viridilucens* on the concentration of phenolic compounds. (**A**) 2,4,6-Trichlorophenol (●), 4-cyanophenol (■), and 4-nitrophenol (▲). (**B**) Phenol (○), 4-chlorophenol (□), and 4-methoxyphenol (△). Error bars represent standard deviation of triplicates.

(Miyazaki et al. 2002). The higher the value of log K_{OW}, the more toxic the phenolic compound is to the studied species. However, K_{OW} as single descriptor (and this is even worse in the case of weak organic acids such as phenolic compounds) does not accurately describe the biological membrane (Escher and Schwarzenbach 2002). For some organisms, K_{lipw} is a better descriptor for the interaction of organic toxicants with the biological membrane than log K_{OW} (Escher and Schwarzenbach 2002).

We used 2 simple and common parameters, pK_a and K_{OW}, to predict the toxicity of phenolic compounds to the fungus *G. viridilucens* based on EC50 values. Values of pK_a and K_{OW} were obtained from the literature (Table 1; Nowosielski and Fein 1998; Li and Hoffman 1999; Jäntschi and Bolboacă 2007). Only the bulky and less polar 2,4,6-trichorophenol varied within the pH of agar medium (pH 5.5–6.0). Therefore, the reported experimental value of K_{OW} for 2,4,6-trichlorophenol at pH 6.1 was used (Nowosielski and Fein 1998). Ionization was negligible for the other phenolic compounds at pH 6, and so the calculated values of K_{OW} were utilized (Jäntschi and Bolboacă 2007). Univariate and multivariate linear regressions were determined to characterize steric and electronic effects of substituted phenolic compounds in their toxicity.

TABLE 1: Physicochemical parameters of phenolic compounds and the median effect concentration obtained from the bioluminescent assay with the fungus *Gerronema viridilucens*

Phenol	pK_a[a]	Log K_{OW}[b]	EC50 (mM)[c]
2,4,6-Trichorophenol	5.99	3.76	0.14 ± 0.01
4-Nitrophenol	7.16	1.91	0.30 ± 0.02
4-Cyanophenol	7.95	1.60	0.7 ± 0.1
Phenol	9.98	1.49	0.97 ± 0.05
4-Chlorophenol	9.37	2.39	2.0 ± 0.2
4-Methoxyphenol	10.20	1.34	5.0 ± 0.5

[a]Acid dissociation constant (Li and Hoffman 1999).
[b]Calculated 1-octanol/water partition coefficient (Jäntschi and Bolboacă 2007), except for 2,4,6-trichlorophenol, which was determined experimentally at pH 6.1 (Nowosielski and Fein 1998).
[c]Median effect concentration (EC50) and standard deviation values were obtained from curves depicted in Figure 1 and fitted using Equation 1. The data correspond to the values shown in the Supplemental Data, Table S3, divided by 6, which is the dilution factor of phenolic compounds in agar medium.

The univariate linear correlation between log EC50 and pK_a (adjusted $r^2 = 0.81$) was stronger than that with log K_{OW} (adjusted $r^2 = 0.44$). This result was unexpected, because univariate models using log K_{OW} have been used successfully to predict the toxicity of phenolic compounds to very different organisms (Arold et al. 1988; Miyazaki et al. 2002). It was also verified whether the correlation between log EC50 and log K_{lipw} (Escher and Schwarzenbach 2002) was stronger than with log K_{OW}, by using log K_{OW} (Table 1) and an equation described for polar chemicals ($\log K_{lipw} = 0.904 \times \log K_{OW} + 0.515$; Vaes et al. 1997). However, a weaker correlation with log K_{lipw} was obtained compared with log K_{OW} (adjusted $r^2 = 0.31$; Table 2). Hence, even using the lipid/water partition coefficient it was not possible to obtain a strong univariate correlation with some parameters related to the lipophilicity of the phenolic compound.

The medium pH and pK_a of phenolic compounds are reportedly as important as log K_{OW} to describe toxicity (Arold et al. 1988; Zhao et al. 2009). Accordingly, the strongest correlation with log EC50 in the present study was described by the bivariate regression with pK_a and log K_{OW} (adjusted $r^2 = 0.94$; Figure 2 and Table 2).

Although information on the toxicity of phenolic compounds to basidiomycetes is scarce in the literature, there are many reports on the toxicity of chlorophenols and nitrophenols to bacteria and aquatic and terrestrial organisms. The toxicity of 19 chlorophenols to 7 classes of organisms, including 2 bacteria, 15 fungi, 1 microcrustacean, and 2 fishes, has been predicted (Arold et al. 1988; Zhao et al. 2009). The mechanism of their toxicity can be explained in terms of narcosis and/or uncoupling of the oxidative phosphorylation (Escher and Schwarzenbach 2002; Jäntschi and Bolboacă 2007). Narcosis is conceived of as a nonspecific, reversible mode of toxicant

TABLE 2: Univariate and bivariate linear regressions obtained from the correlation of physicochemical parameters of phenolic compounds (pK_a, K_{OW}, and K_{lipw}) with log EC50 values from the bioluminescent assay with the fungus *Gerronema viridilucens*[a]

Log EC50	Adj. R^2	SD	No.
$= -(5.7 \pm 0.6) + (0.30 \pm 0.06)pK_a$	0.81	0.24	6
$= -(2.2 \pm 0.5) - (0.4 \pm 0.2)\log K_{OW}$	0.44	0.47	6
$= -(0.5 \pm 0.3) - (2.0 \pm 0.6)\log K_{lipw}$	0.31	0.46	6
$= -(4.4 \pm 0.5) - (0.10 \pm 0.07)\log K_{OW} + (0.16 \pm 0.04)pK_a$	0.94	—	6

[a]Univariate and multivariate linear fitting was obtained by using the linear regression models as implemented in Origin 2016 software. Standard deviations (SDs) were not used as weight in linear fittings.
K_{OW} = the 1-octanol/water partition coefficient; K_{lipw} = the lipid/water partition coefficient; pK_a = the acid dissociation constant.

action, which causes generalized decrease in biological activity (Rand 1995).

As just discussed, log EC50 presented stronger univariate correlations with pK_a and stronger bivariate correlations with pK_a and $\log K_{OW}$. Univariate regression with either $\log K_{OW}$ or $\log K_{lipw}$ is not sufficient to describe the toxicity of the phenolic compound series we investigated. The results obtained strongly suggest that the toxicity profile of phenolic compounds can be explained by the classical protonophoric action of weakly acidic uncouplers on the interface of the mitochondrial membrane (Terada 1990). Potent uncouplers typically contain a weakly acidic group, such as the classical 2,4-dinitrophenol, bearing electron-withdrawing substituents and/or bulky hydrophobic groups. These features are very suitable to describe highly toxic and lethal 2,4,6-trichlorophenol, the compound that exhibited the highest toxicity to the fungus. In this regard, the uncoupling effect of phenolic compounds can be predicted by a binomial linear correlation with $\log K_{OW}$ and pK_a (Yarden and Osherov 2010). The uncoupling mechanism for adenosine triphosphate (ATP) biosynthesis depends on the stability of the phenolate-moiety in the hydrophobic portion of the mitochondrial membrane (Terada 1990). The presence of bulky moieties in the phenolic compound can increase hydrophobicity and occlude the ionic charge from the medium. This process can unbalance the mitochondrial complexes in the matrix and consequently impair ATP production (Terada 1990).

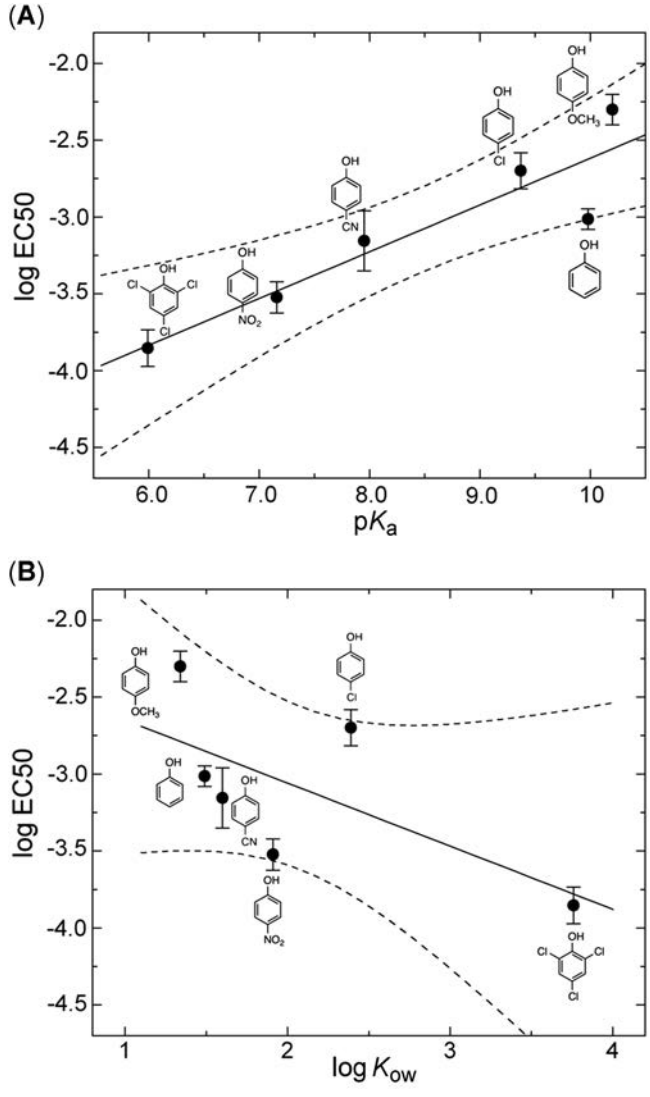

FIGURE 2: Univariate dependence of the median effective concentration (log EC50) values obtained from the bioluminescent assay with the fungus *Gerronema viridilucens* on pK_a (**A**) and $\log K_{OW}$ at pH 6.1 (**B**) of phenolic compounds. Standard deviations were not used as weight in linear regressions. Dashed lines are 95% confidence limits.

Also worth noting is that recent results obtained from investigation of the biosynthetic mechanism of hispidin, the precursor of fungal luciferin, revealed its dependence on coupled ATP pyrophosphate cleavage (Kaskova et al. 2017; Kotlobay et al. 2018). In the proposed mechanism for fungal bioluminescence (Figure 3), caffeic acid (originating from the Shikimate pathway) is first converted in the presence of *hispidin synthase* (*HispS*) into hispidin, consuming cofactors including ATP. The second step involves the hydroxylation of hispidin by *hispidin-3-hydroxylase* (*H3H*), yielding 3-hydroxyhispidin (luciferin), whose oxidation by *luciferase* (*LUZ*) leads to light emission and the production of caffeylpyruvic acid (oxyluciferin). Finally, oxyluciferin regenerates caffeic acid in the presence of *caffeylpyruvate hydroxylase* (*CPH*). Hence, a decrease in ATP output in the cell caused by phenolic compound–induced mitochondrial uncoupling expectedly hinders the biosynthesis of fungal luciferin and then light emission intensity. It is well known that aerobic organisms depend on mitochondrial production of ATP to support vital metabolic pathways, which are ultimately responsible for sustaining life. To accomplish these processes, the mobilization of ATP and the consumption of molecular oxygen must be well balanced; otherwise adverse outcomes or metabolic compensation may occur. In this sense, we believe that the bioassay

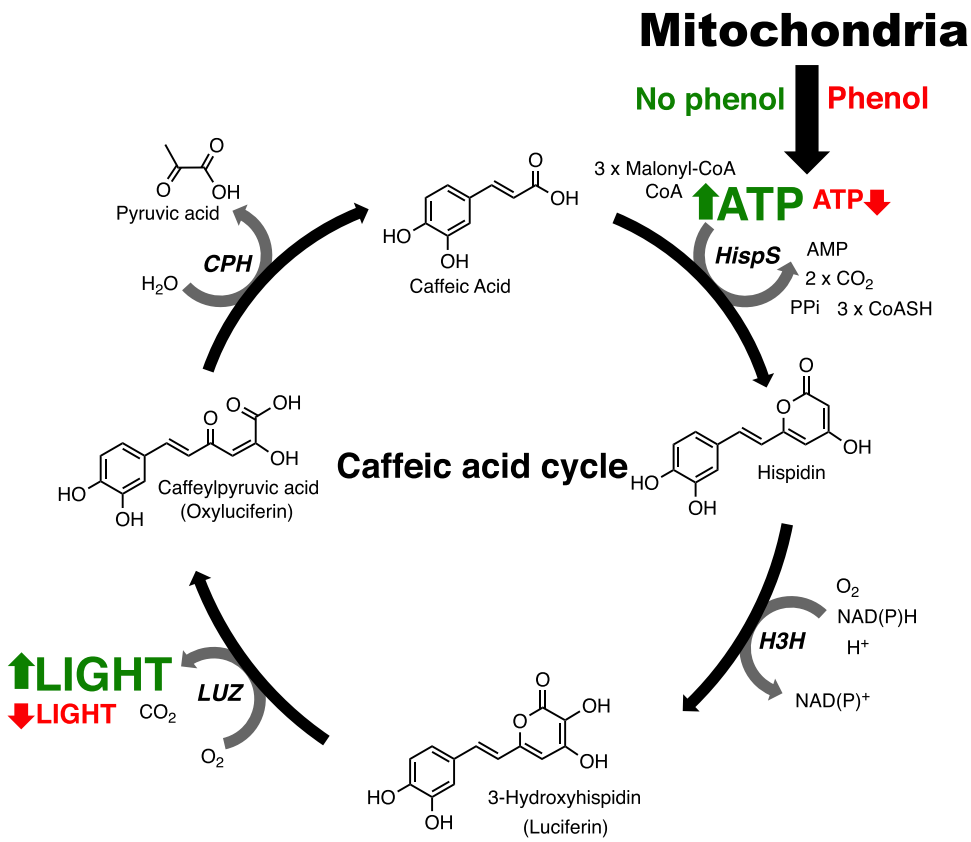

FIGURE 3: Schematic representation of phenol-induced mitochondrial impairment of ATP biosynthesis and its effect on the caffeic acid cycle (Kaskova et al. 2017; Kotlobay et al. 2018), involved in fungal bioluminescence. In absence of phenol (green), ATP output and light emission are higher. The exposure to phenol (red) decreases ATP and fungal bioluminescence. *CPH* = *caffeylpyruvate hydroxylase*; *HispS* = *hispidin synthase*; PPi = pyrophosphate; CoASH = coenzyme A; NAD(P)H = reduced nicotinamide adenide dinucleotide or reduced nicotinamide adenide dinucleotide phosphate; H3H = hispidin-3-hydroxylase; *LUZ* = *luciferase*.

and correlations we present are useful tools to signal early damage to the fungal system induced by phenolic compounds.

Basidiomycetes are eukaryote organisms, and thus it is likely that phenolic compounds present the same toxic mode of action that is displayed in other organisms, once they uncouple the mitochondrial ATP biosynthesis, and therefore the cellular energetic balance. However, few studies have been dedicated to exploring the toxicity of organic compounds to the Fungi Kingdom, which is of utmost importance to shed more light on how the soil ecosystem survives in the industrial world. Basidiomycetes such as *G. viridilucens* are essential to recycle the cellulose from decaying plants to other organisms along the terrestrial food chain. Without them, cellulose would be stuck in its natural form and would eventually impact nutrient availability for the soil microfauna and microflora.

CONCLUSIONS

We evaluated the toxicity of a series of phenolic compounds using a basidiomycete bioluminescent fungus. Bioluminescence intensity was demonstrated to be a suitable and precocious endpoint to underpin and rank the toxicity of phenolic compounds with different degrees of acidity and hydrophobicity. Although the pK_a of phenolic compounds can be used alone to predict their toxicity, the bivariate linear regression combined with log K_{OW} was shown to be even better. These findings, together with the highest toxicity observed with 2,4,6-trichlorphenol, a bulky, hydrophobic, and weakly acidic phenolic compound, indicate that the mechanism of the fungal action is likely to involve the classical and well-studied uncoupling of ATP biosynthesis in mitochondria. The biosynthesis of hispidin—the precursor of fungal luciferin—depends on ATP, and thus the lower the availability of ATP, the lower the bioluminescence. The present study addresses a significant gap of data on the toxicity of environmentally relevant organic compounds such as phenolic compounds to basidiomycetes, thereby resulting in an imbalance in the soil food traffic essential for living organisms.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4740.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (stevani@iq.usp.br).

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