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Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from *Pleurotus ostreatus*

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Recommended Citation

Chapple, Alexander; Nguyen, Luong; Hai, Faisal I.; Dosseto, Anthony; Rashid, Md. Harun-Or; Oh, Seungdae; Price, William E.; and Nghiem, Long D., "Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from *Pleurotus ostreatus*" (2017). *Faculty of Engineering and Information Sciences - Papers: Part B*. 985.
<https://ro.uow.edu.au/eispapers1/985>

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Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from *Pleurotus ostreatus*

Abstract

This study investigated the influence of inorganic salts on enzymatic activity and the removal of trace organic contaminants (TrOCs) by crude laccase from the white-rot fungus *Pleurotus ostreatus*. A systematic analysis of 15 cations and anions from common inorganic salts was presented. Laccase activity was not inhibited by monovalent cations (i.e. Na^+ , NH_4^+ , K^+), while the presence of divalent and trivalent cations showed variable impact - from negligible to complete inhibition - of both laccase activity and its TrOC removal performance. Of interest was the observation of discrepancy between residual laccase activity and TrOC removal in the presence of some ions. Mg^{2+} had negligible impact on residual laccase activity but significant impact on TrOC removal. Conversely, F^- showed greater impact on residual laccase activity than on TrOC removal. This observation indicated different impacts of the interfering ions on the interaction between laccase and TrOCs as compared to that between laccase and the reagent used to measure its activity, implicating that residual laccase activity may not always be an accurate indicator of TrOC removal. The degree of impact of halides was in the order of $\text{F}^- > \text{I}^- > \text{Br}^- > \text{Cl}^-$. Particularly, the tolerance of the tested laccase to Cl^- has important implications for a range of industrial applications.

Disciplines

Engineering | Science and Technology Studies

Publication Details

Chapple, A., Nguyen, L. N., Hai, F. I., Dosseto, A., Rashid, M., Oh, S., Price, W. E. & Nghiem, L. D. (2019). Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from *Pleurotus ostreatus*. *Biocatalysis and Biotransformation*, 37 (1), 10-17.

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2 **extracellular enzyme from *Pleurotus ostreatus***

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4 **Revised Manuscript Submitted to**
5 **Biocatalysis and Biotransformation**
6 **November 2017**

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26 **Abstract:**

27 This study investigated the influence of inorganic dissolved constituents (i.e., cations and anions)
28 on enzymatic activity and trace organic contaminants (TrOCs) removal by crude laccase from the
29 white-rot fungus *Pleurotus ostreatus*. A systematic analysis of 15 cations and anions from
30 common inorganic salts was presented. Laccase activity was not inhibited by monovalent cations
31 (i.e., Na⁺, NH₄⁺, K⁺), while the presence of divalent and trivalent cations showed variable impact –
32 from negligible to complete inhibition – of both laccase activity and its TrOC removal
33 performance. Of interest was the observation of discrepancy between residual laccase activity and
34 TrOC removal in the presence of some ions. Mg²⁺ had negligible impact on residual laccase
35 activity but significant impact on TrOC removal. Conversely, F⁻ showed greater impact on
36 residual laccase activity than on TrOC removal. This observation indicated different interactions
37 of the interfering ions with laccase and TrOCs as compared to laccase and the reagent used to
38 measure its activity, meaning that residual laccase activity may not always be an accurate indicator
39 of TrOC removal. The degree of impact of halides was in the order of F⁻ > I⁻ > Br⁻ > Cl⁻.
40 Particularly, the tolerance of the tested laccase to Cl⁻ has important implications for a range of
41 industrial applications.

42 **Keywords:** Laccase; trace organic contaminants (TrOCs); inorganic salts; halides; metals

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56 **Introduction**

57 Trace organic contaminants (TrOCs) include, among others, pharmaceuticals and industrial
58 chemicals. (Hoeger et al. 2005; Lee et al. 2014; Luo et al. 2014). Conventional wastewater
59 treatment processes are not designed to remove TrOCs and thus wastewater treatment plant
60 effluent is considered as a major source of TrOC into the environment. TrOCs often occur in
61 various aquatic environment at concentrations ranging from few ng/L to µg/L (Luo et al. 2014).
62 Due to the persistence and/or bioaccumulative properties, many of the TrOCs pose risk to
63 ecosystem and public health.

64 Enzymatic transformation of TrOCs has recently attracted much attention as a promising
65 eco-friendly concept (Yang et al. 2013). Laccase (Benzenediol: oxygen oxidoreductase; EC
66 1.10.3.2) is of interest as it only requires molecular oxygen as a co-substrate, unlike the
67 peroxidases which require H₂O₂. Laccase catalysed oxidation mechanism includes the reduction
68 of molecular oxygen to water and one electron oxidation of various aromatic substrates such as
69 TrOCs (Majeau et al. 2010; Nguyen et al. 2014b; Yang et al. 2013). Laccase has been found to
70 be abundant in many white-rot fungi. The potential of laccase for the removal of TrOCs has been
71 investigated intensively by various researchers. Results have demonstrated that laccase can
72 effectively degrade a range of TrOCs (Majeau et al. 2010; Nguyen et al. 2014b; **Spina et al.**
73 **2015**; Tran et al. 2010; Yang et al. 2013). TrOC removal by laccase is governed by factors such
74 as pH, temperature, and physicochemical properties of TrOCs (Asif et al. 2017a). For example,
75 optimum pH for the removal of triclosan was in the range of 5 to 5.5 (Kim & Nicell 2006b),
76 while diclofenac was found to be removed at the highest rate under acidic conditions (pH 3 - 4.5)
77 (Nguyen et al. 2014a). TrOC properties can also strongly influence laccase performance. The
78 compounds which contain phenolic moiety are more amendable to laccase. On the other hand,
79 compounds which contains functional group such as carboxylic, amide, and chloride are resistant
80 to laccase oxidation (Asif et al. 2017b; **Shi et al. 2017**; Tran et al. 2010; Yang et al. 2013).

81 Only a few studies have provided some insight into the effect of heavy metals on the
82 removal of dye by laccase (Murugesan et al. 2009; Rodríguez Couto et al. 2005). Murugesan et
83 al. (2009) reported that metal ions such as Ca²⁺, Co²⁺, Cu²⁺ and Zn²⁺ at a concentration of 1 mM
84 did not have impact on laccase performance. However, it is hypothesized that some ions may
85 block or interfere with the active sites of laccase and thus decrease its activity (Asif et al. 2017b;
86 Tran et al. 2010; Yang et al. 2013). Notwithstanding the available studies, the impact of

87 wastewater-derived dissolved interfering compounds on the removal of TrOCs by laccase has not
88 been fully elucidated. Dissolved organic (e.g., humic substance, organic matters) and inorganic
89 constituents (cations and anions) widely occur in water and wastewater. Therefore, to fully
90 uncover the potential of laccase for the removal of TrOCs, the effects of these constituent ions
91 need to be studied.

92 The aim of this study was to investigate the impact that a range of dissolved inorganic
93 ions impose on laccase including its activity level and its removal of two TrOCs, namely,
94 bisphenol A (BPA) and diclofenac (DCF). The experiment will cover a range of common salts at
95 different concentrations, with the objective of not only representing wastewater streams that may
96 be encountered, but also gaining an understanding of relative ionic influences from which
97 extrapolations can be made to predict the influence of certain wastewater components. The
98 results will allow development of enzyme based treatment systems to be optimised, especially
99 around TrOC removal.

100 **Materials and Methods**

101 *Materials*

102 *TrOCs and dissolved interfering salts*

103 Two TrOCs, namely, bisphenol A (BPA) and diclofenac (DCF) (Sigma–Aldrich, USA) were
104 selected in this study because of their ubiquitous presence in wastewater and wastewater-
105 impacted waterbodies.

106 A set of cations and anions, which occur widely in water and wastewater, were selected
107 to test their impact on laccase activity and its TrOC removal performance. Table 1 presents the
108 selected cations and anions and the associated original salts.

109 *Crude laccase preparation*

110 A white-rot fungus *Pleurotus ostreatus* (ATCC 34675) was incubated in malt extract broth (2
111 g/L) at a pH of 4.5 to produce crude enzyme solution. The culture was kept in a rotary shaker at
112 28 °C and 70 rpm for 5 days. The crude enzyme was obtained by decanting the liquid portion
113 into a sterile container. Under these culture conditions, the enzyme preparation exhibited
114 predominantly laccase activity (70 to 90 $\mu\text{M}_{(\text{DMP})}/\text{min}$) and negligible peroxidase activity. The
115 crude laccase preparation thus obtained was stored at 4 °C until use.

116 ***Batch test description***

117 A strategic experimental plan was implemented in this study. Kim and Nicell (2006a), reported
118 negligible impact of Na⁺ and NH₄⁺ on laccase activity. Therefore, at first, SO₄²⁻ dissolved from
119 sodium and ammonium sulfate salts was tested to elucidate if SO₄²⁻ has low impact so that
120 sulfate salts can be used as a source of cations. After confirming the low impact of SO₄²⁻, a range
121 of sulfate salts (Table 1) was used.

122 To test the variance in influence of the anions, tests were conducted to include PO₄³⁻ and
123 NO₃⁻, and four halides i.e., F⁻, I⁻, Br⁻ and Cl⁻. The anions were matched with either Na⁺ or K⁺ to
124 select the salt to be added to the test solution (Table 1).

125 The test solution was prepared in sterile test tubes. The impact of each ion was tested at
126 the following concentrations: 1, 10, 100 and 250 mM. TrOCs were each added at a nominal
127 concentration of 100 µg L⁻¹ (actual measured concentrations of 116 ± 10 µg/L and 109 ± 5 µg/L (n
128 = 14) of BPA and DCF, respectively).

129 A set of control tubes were prepared in the same fashion but excluding the interfering
130 cations and anions. The test tubes were capped and incubated in a rotary shaker at 70 rpm and 25
131 °C for 24 h, following which the residual laccase activity and TrOC concentration were
132 measured.

133 ***Analytical methods***

134 *Laccase activity*

135 Laccase activity was measured by monitoring the change in absorbance at 468 nm due to the
136 oxidation of 2,6-dimethoxy phenol (DMP) at room temperature over 2 min using a
137 spectrophotometer (Spec UV-1700, Shimadzu, Kyoto, Japan) (Hai et al. 2009). Laccase activity
138 was calculated from the molar extinction coefficient $\epsilon = 49.6 \text{ /mM.cm}$ and expressed in µM
139 substrate/min. Lignin and manganese peroxidase activity were determined as described
140 elsewhere (Camarero et al. 1999).

141 *TrOC analysis*

142 A HPLC system (Shimadzu, Kyoto, Japan), equipped with a Supelco Drug Discovery
143 300 × 4.6 mm C-18 column (5 µm pore size) and a UV-vis detector, was used to measure the
144 TrOC concentrations. The detection wavelength was 280 nm and the column temperature was
145 20 °C. The sample injection volume was 50 µL. The mobile phase comprised acetonitrile and

146 Milli-Q water buffered with 25 mM KH_2PO_4 (pH = 4.8). Two eluents, A (80%
147 acetonitrile + 20% buffer, v/v) and B (20% acetonitrile + 80% buffer, v/v) were delivered at
148 0.7 mL/min through the column for 30 min in the following time-dependent gradient
149 proportions: [Time (min), % of B] = [0, 80], [12, 80], [20, 0], [25, 0], [25, 80]. Under the
150 operating conditions, the retention time of BPA and DCF was 23 and 26 min, respectively. The
151 limit of quantification for the analytes under investigation using these conditions was
152 approximately 10 $\mu\text{g/L}$. HPLC samples were prepared by diluting the samples two-fold by
153 adding methanol to immediately stop any residual enzyme activity in the sample (Nguyen et al.
154 2014a).

155 **Results and discussion**

156 *Impact of cations*

157 Kim and Nicell (2006a) reported that sodium and ammonium ions had negligible impact on
158 laccase activity. Therefore, sodium and ammonium sulfate salts were tested first to elucidate the
159 impact of SO_4^{2-} . A negligible impact on laccase activity was observed until a SO_4^{2-} concentration
160 of 250 mM, where a 23% drop in activity was observed. At this concentration, DCF removal
161 efficiency showed a 30% decrease compared to the control (Figure 1). Based on this initial
162 assessment of the impact of SO_4^{2-} , monovalent, divalent, and trivalent cations dissolved from
163 sulfate salts were evaluated for their impact on laccase activity and TrOC removal performance.

164 [FIGURE 1]

165 *Impact of type of cations*

166 Figure 2 illustrates the impact of cations on both laccase activity and its performance on TrOC
167 removal. The results show that monovalent cations (Na^+ , NH_4^+ , and K^+) have low impact on
168 both laccase activity and TrOC removal. At a concentration of 250 mM, Na^+ and NH_4^+ caused a
169 20% drop in laccase activity, whereas, K^+ showed positive impact with about 5% increment in
170 observed laccase activity. Further discussion regarding this apparent increase in laccase activity
171 is available in the penultimate section of the paper. The relative stability of laccase was reflected
172 in the removal of BPA. On the other hand, Na^+ and NH_4^+ showed 50% and 30% decrease in DCF
173 removal, respectively. Comparatively less impact of cations on BPA removal can be attributed to
174 the fact that it is a phenolic compound which is highly amenable to laccase-catalysed degradation
175 (Spina et al. 2015; Yang et al. 2013).

176 Divalent and trivalent cations (Mg^{2+} , Ca^{2+} , Cu^{2+} , Zn^{2+} , Mn^{2+} and Al^{3+}) showed variable
177 impact on laccase activity and TrOC removal. Mg^{2+} did not adversely affect laccase activity but
178 showed significant impact on TrOC removal: 70 and 60% decreases in BPA and DCF removal,
179 respectively was observed. It is possible that Mg^{2+} inhibited the catalytic activity of laccase on
180 BPA and DCF but not on DMP (the substrate used in enzyme assay). The mismatch between
181 impact on residual laccase activity and TrOC removal suggests that residual laccase activity may
182 not be always used to indicate the impact of interfering cations on laccase-catalysed TrOC
183 removal.

184 Ca^{2+} and Cu^{2+} showed significant impact on both laccase activity and TrOC removal. Up
185 to 93% inhibition of laccase activity was observed in the presence of Cu^{2+} . This was
186 accompanied by 90% and 70% drop in removal of BPA and DCF, respectively. This observation
187 is consistent with that in the available literature. For example, Cu^{2+} has been shown to impose
188 significant influence on laccase even at low concentrations (Hou et al. 2014; Lorenzo et al.
189 2005). Murugesan et al., (2009) reported a severe impact of Cu^{2+} on laccase from *Ganoderma*
190 *lucidum*.

191 Zn^{2+} and Mn^{2+} demonstrated a moderate impact on both laccase activity and TrOC
192 removal. Zn^{2+} caused about 30% and 40% reduction in laccase activity and TrOC removal,
193 respectively. A stronger impact of Al^{3+} laccase activity and TrOC removal was noted (Figure 2).
194 To date the mechanisms in which cations affect laccase activity and its TrOC removal
195 performance have not been elucidated. Some possible mechanisms of metal-induced inactivation
196 of laccase include modification of amino acid residue on enzyme, copper chelation or
197 conformational change of the enzymes (Chmelová & Ondrejovič 2015; Johannes & Majcherczyk
198 2000).

199
200

[FIGURE 2]

201 *Impact of cation concentrations*

202 The cations selected in this study frequently occur in water and wastewater. Their concentration
203 can vary with season, geographical location and type of water/wastewater. Therefore, the
204 stability of laccase and its TrOC removal performance against several different concentrations
205 (1-250 mM) of cations was assessed in this study (Figure 3).

206 As discussed before, the monovalent cations *i.e.*, Na⁺, NH₄⁺, and K⁺ showed little impact
207 on laccase activity over the whole concentration range (Figure 3). A previous study by Shankar
208 et al. (2015) also reported no impact of Na⁺ (0.5 - 15 mM) on laccase from *Peniophora sp.* On
209 the other hand, Trovaslet et al. (2007) found that with an increase in Na⁺ concentration from 0 –
210 1 M, the activity of the laccase from *T. versicolor* gradually decreases from 100% to 50%. Our
211 results demonstrate that up to a concentration of 250 mM, laccase from *P. ostreatus* has strong
212 tolerance to monovalent cations such as Na⁺, NH₄⁺, and K⁺. Similar to laccase activity, the
213 change in Na⁺, NH₄⁺, and K⁺ concentration did not show any considerable impact on BPA
214 removal. However, approximately 40-50% inhibition of DCF removal was observed at all tested
215 concentrations of the monovalent ions Na⁺, NH₄⁺, and K⁺, demonstrating again the resistance of
216 DCF to laccase-catalysed degradation.

217 [FIGURE 3]

218 The divalent cations showed a strong effect at a concentration of 250 mM (Figure 2).
219 Therefore, it was interesting to observe their impacts over a lower concentration range. The
220 residual laccase activity in the presence of Mg²⁺ appear to be higher than that of the control.
221 Mg²⁺ was also found to increase laccase activity in a study by Shankar and Nill (2015). Further
222 discussion regarding this apparent increase in laccase activity is available in the penultimate
223 section of the paper.

224 In contrast to laccase activity, the removal of BPA and DCF gradually dropped with
225 Mg²⁺ concentration-increase. Ca²⁺ affected laccase activity at 100 mM. However, the effect of
226 Ca²⁺ on TrOC removal was significant even at 1 mM (Figure 3). Murugesan et al. (2009)
227 observed a concentration-dependent effect of Ca²⁺ on dye decolourisation by laccase from
228 *Ganoderma lucidum*. Our study not only confirms such salt concentration dependent effect on
229 TrOC but also highlights different extents of impact on laccase activity and TrOC removal.

230 Murugesan et al. (2009) observed a minor impact of Zn²⁺ on laccase and its dye
231 decolourisation capacity. Similarly, in the current study, irrespective of its concentration, Zn²⁺
232 showed moderate inhibition of laccase activity and TrOC removal (Figure 3). Mn²⁺ also
233 exhibited a moderate impact on laccase activity with around 20% inhibition at all tested
234 concentrations. It is noteworthy that a 10% increase in BPA removal was achieved in presence of
235 1 and 10 mM Mn²⁺ (Figure 3). This is consistent with a few other studies who report enhanced

236 enzymatic dye decolourisation in presence of metal ions in low concentration range, generally
237 below 15 mM (Majeau et al. 2010; Murugesan et al. 2009; Shankar & Nill 2015).

238 Cu^{2+} is of special interest as it is a key component in laccase structure. Previous studies
239 showed no effect of Cu^{2+} (Murugesan et al. 2009; Shankar & Nill 2015) up to a concentration of
240 15 mM. The observation of significant inhibition at 250 mM in this study (Figure 2) necessitated
241 testing at lower concentration range. As showed in Figure 3, Cu^{2+} has strong impact on both
242 laccase stability and TrOC removal. Even at 1 mM, laccase activity was reduced by 50%. TrOC
243 removal illustrated an interesting response to Cu^{2+} . Throughout this study, compared to BPA,
244 DCF removal was observed to be affected more by different salts. As noted before, this can be
245 explained by the fact that BPA has a phenolic moiety which makes it especially amenable to
246 laccase catalysis (Majeau et al. 2010; Nguyen et al. 2014b; Tran et al. 2010; Yang et al. 2013).
247 However, in the current study, in presence of 1 mM Cu^{2+} , BPA removal was down to 10%
248 compared to control, while DCF removal appeared less affected. It is probably because the
249 interaction between BPA and laccase was affected in the presence of Cu^{2+} , but not that of DCF
250 and laccase. It is also possible that a different mode of interaction is involved here: Cu^{2+} is
251 considered as a pro-oxidant and it can act as a catalytic oxidant which may form a copper-DCF
252 complex, making DCF more amenable to degradation (Yang et al. 2013).

253 ***Impact of anions***

254 *Impact of halides*

255 Halides are found in many industrial products and have been associated with significant
256 environmental pollution and toxicity. Compared to Cl^- and F^- , the impact of halides Br^- and I^- on
257 laccase have received much less attention in recent literature. Thus, the selection of all four
258 halides in this study fills an important research gap.

259 In a similar approach to that taken with using sulfate salts to assess the impact of cations,
260 the halides were matched with cations such as Na^+ or K^+ whose low impact had already been
261 established (Figure 3). Cl^- showed about 20% reduction in laccase activity at a concentration of
262 250 mM (Figure 4). The extent of laccase activity reduction due to sodium chloride was even
263 smaller than that for sodium sulfate (Figure 3). Consistent with laccase stability, Cl^- effect on
264 BPA removal was negligible. However, up to 40% removal of DCF was observed. The effect of
265 Cl^- on laccase stability and its TrOC removal performance has not been thoroughly tested in the

266 literature. Champagne et al. (2013) reported that Cl^- strongly affected both the laccase activity
267 and its dye decolourisation efficiency. However, our study confirms that Cl^- does not impose as
268 high of a negative influence on laccase secreted by *P. ostreatus*.

269 In this study, Br^- showed no significant impact on laccase activity. The activity dropped
270 by 10% at 1 mM, and then levelled off at 20% for 10, 100 and 250 mM. However, BPA removal
271 efficiency gradually decreased with the increase of Br^- concentration. It is noted that the Br^- ion
272 was dissolved from potassium bromide. In comparison with the impact of potassium sulfate
273 (Figure 3), BPA removal decreased more in case of potassium bromide (Figure 4), which
274 confirmed some impact of Br^- . On the other hand, I^- exhibited a strong impact on laccase
275 activity. The residual laccase activity dropped by about 50 % in the presence of 1 mM I^- and then
276 reduced to 16% at higher concentrations. However, only about 30% reduction in BPA removal
277 was observed at the highest I^- concentration i.e., 250 mM. This study is the first to report the
278 impact of Br^- and I^- on laccase stability and its TrOC removal performance.

279 F^- inhibited laccase activity significantly (Figure 4): the activity reduced by 50% and
280 95% at F^- concentrations of 1 mM and 10 mM, respectively. At 100 and 250 mM, no laccase
281 activity was detected, which highlights the magnitude of the influence of F^- . From literature, a
282 complete inhibition of enzymatic activity can be seen at F^- levels as low as 1 mM (Jung et al.
283 2002), but the available studies did not investigate TrOC removal. The current study additionally
284 confirms that F^- strongly affects the laccase-catalysed degradation of both BPA and DCF.

285 In this study, the halides were observed to have an impact on laccase activity and TrOC
286 removal in the following order: $\text{F}^- > \text{I}^- > \text{Br}^- > \text{Cl}^-$. The difference in the extent of inhibition by
287 halides is probably due to the different mechanisms in which each halide interact with laccase.
288 Morozova et al. (2007) suggested that these anions bind with the Type 2 and 3 copper atoms of
289 laccase, preventing the electron to transfer from the Type 1 site, consequently inhibiting the
290 oxidation pathway. Farnet et al. (2008) reported that Cl^- and Br^- ions act as competitive inhibitors
291 with the electron donor while F^- acts as a non-competitive inhibitor.

292 [FIGURE 4]

293 *Impact of PO_4^{3-} and NO_3^-*

294 PO_4^{3-} and NO_3^- are commonly present in wastewater and wastewater-impacted natural
295 waterbodies. N and P-species are responsible for eutrophication and other environmental
296 hazards. Thus, their impact on laccase activity was tested in this study. Sodium phosphate and

297 sodium nitrate were used to assess the impact of PO_4^{3-} and NO_3^- , respectively as the effect of Na^+
298 was shown to be low (Figure 3). Kim and Nicell (2006a) reported insignificant impact of NO_3^- at
299 low concentrations (around 1-2 mM) on laccase from *T. versicolor*. By investigating a broader
300 range of concentration, our study confirms that the impact of both NO_3^- and PO_4^{3-} on laccase
301 activity can be significant at higher concentrations (Figure 5).

302 [FIGURE 5]

303 *Laccase activity vs TrOC removal performance*

304 There are several key observations made from this study that may be critical in implementing
305 laccase treatment for TrOC removal. The first observation is the discrepancy between the
306 impacts of the salts on laccase activity and the specific TrOC removal in some cases. This
307 observation can be highlighted with the comparison of two sets of results, those of Mg^{2+} and F^- .
308 Mg^{2+} exhibited stable residual enzymatic activity over the 1-250 mM range, but suffered from a
309 70 and 60 % drop in removal efficiency for BPA and DCF, respectively (Figure 3). In this case
310 the laccase remains active, but the mechanism used to oxidise the target contaminant is
311 compromised. This is contrasted with the behaviour of F^- , which showed negligible residual
312 enzyme activity at 250 mM, but still managed to achieve 35 and 11% removal of BPA and DCF,
313 respectively. This emphasizes the different ways in which each of the ions interact and
314 potentially affect laccase activity.

315 [FIGURE 6]

316 The second observation is the elevated residual laccase activity in the presence of some
317 ions (Figure 6). Increased activity in whole-cell preparation in presence of some metals is well
318 established, but there have only been a few mentions of increasing the activity of the isolated
319 laccase by the addition of metal salts. For example, Shankar & Nill. (2015) reported an
320 enhancement of residual laccase activity in the presence of Ca^{2+} , Co^{2+} and Mg^{2+} . When testing
321 CuSO_4 and MnSO_4 , Farnet et al. (2008) observed an apparent increase in enzyme activity for
322 Cu^{2+} (1 mM) and Mn^{2+} (20 mM). It is possible that the salts could increase the solubility of the
323 substrate used for laccase activity measurement (Farnet et al. 2008), therefore increasing its
324 exposure to laccase, allowing greater oxidation.

325 **Conclusion**

326 The inorganic salts evaluated in this study help isolating the relative impact of a range of ions on
327 laccase from *Pleurotus ostreatus*. A variable impact on laccase and its TrOC removal
328 performance was observed. Monovalent cations such as Na⁺, NH₄⁺ and K⁺ had no or low impact
329 on laccase activity and TrOC removal at all the tested concentrations, indicating strong tolerance
330 of this laccase. On the other hand, divalent and trivalent cations showed different degree of
331 influence. Specific halides also had different impacts on laccase performance: the degree of
332 impact was in the order of F⁻ > I⁻ > Br⁻ > Cl⁻. In particular, the tolerance of the tested laccase to
333 Cl⁻ has important implications for a range of industrial applications.

334 **Disclosure of conflict of interest**

335 The authors report no conflicts of interest.

336 **Funding**

337 This study was partially funded by the GeoQuEST Research Centre, UOW, Australia. A part of
338 this study was conducted during Dr Nguyen's career launch fellowship at UOW. Dr Nguyen also
339 acknowledges a postdoctoral fellowship at the Nanyang Technological University, Singapore.

340 **References**

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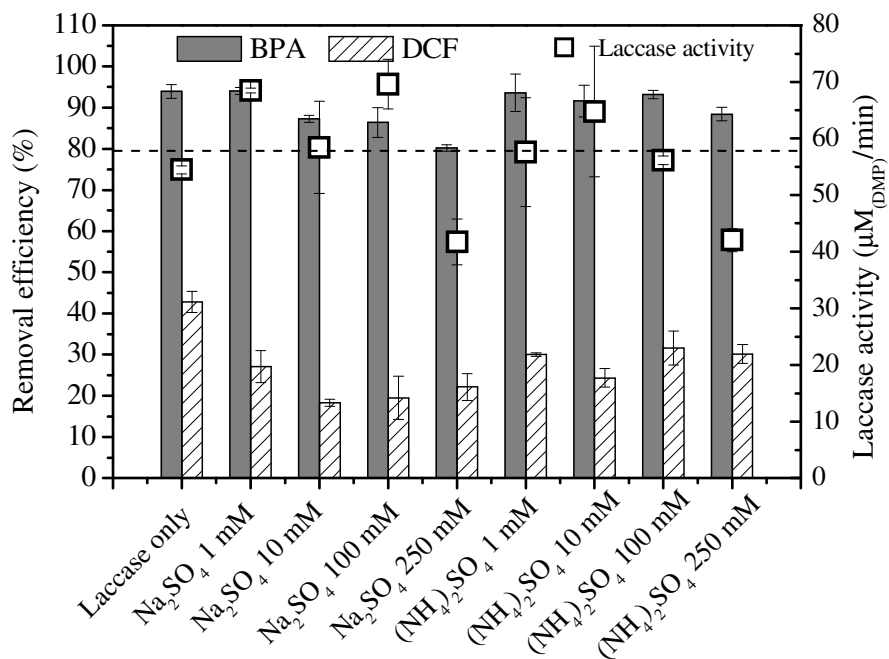


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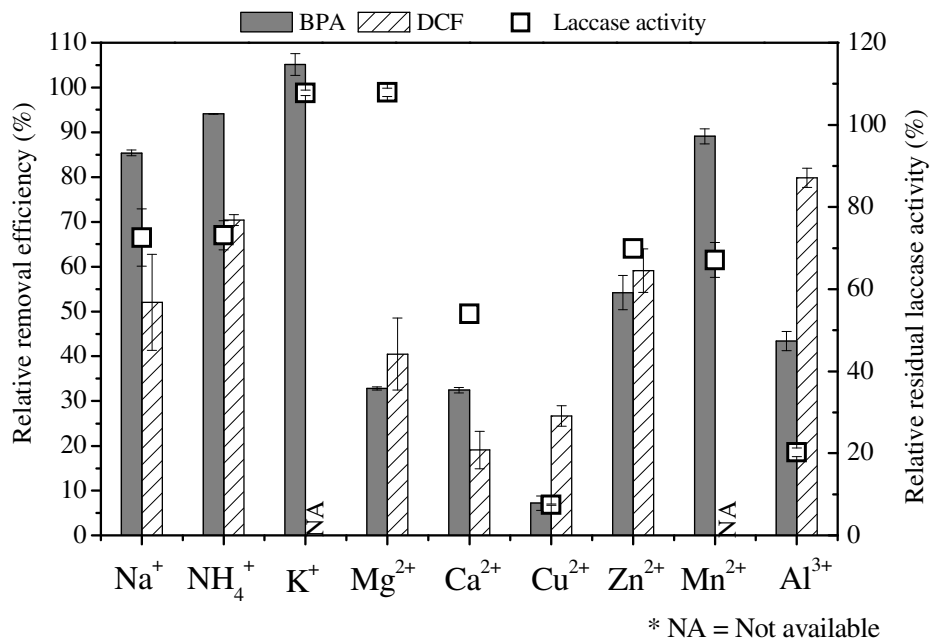


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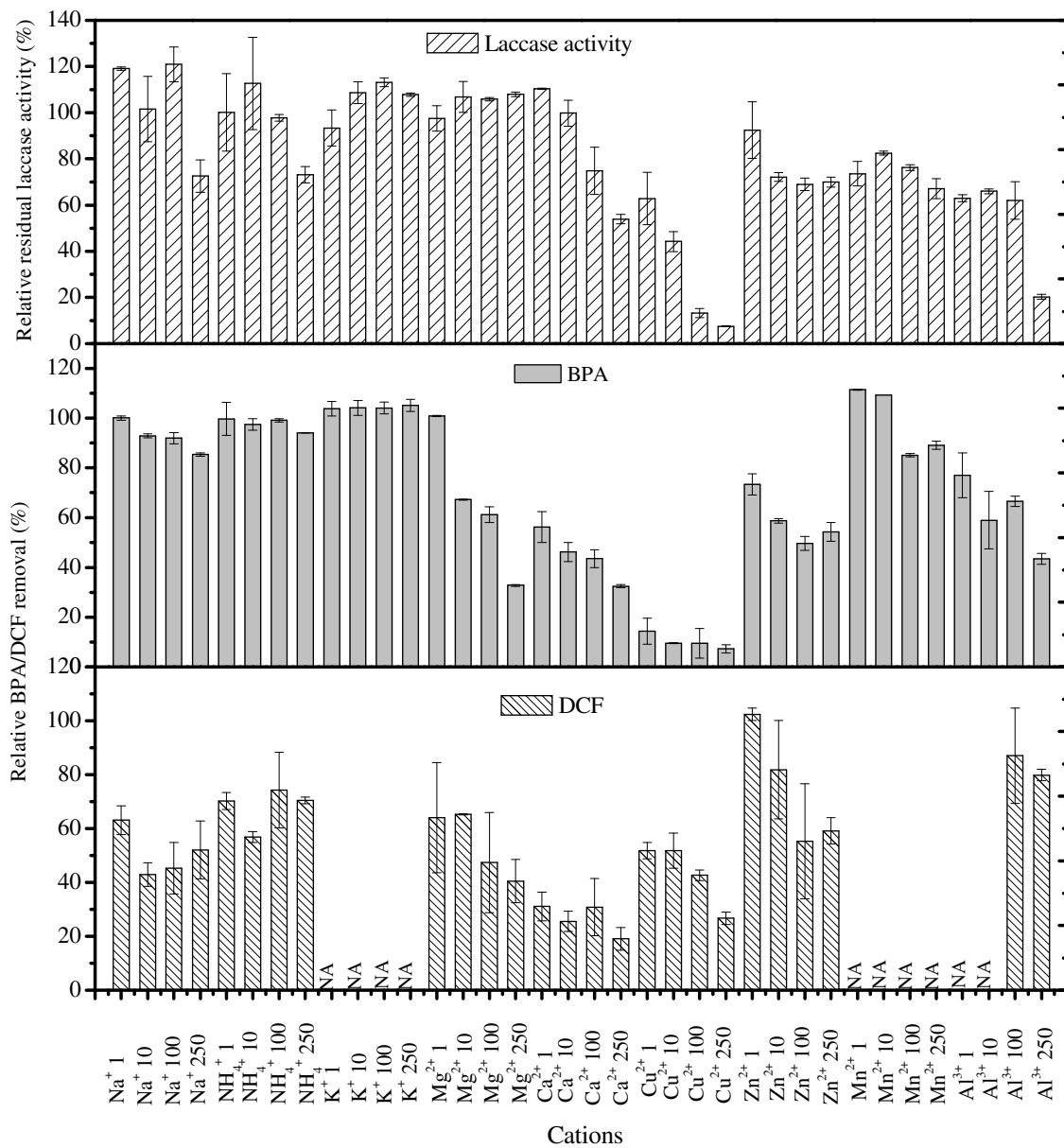


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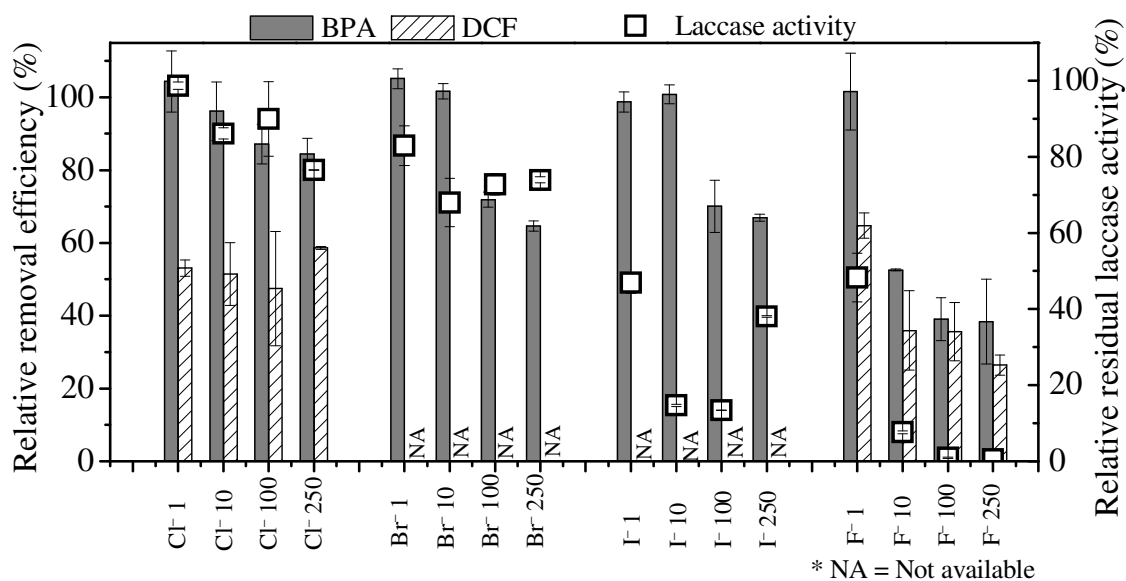


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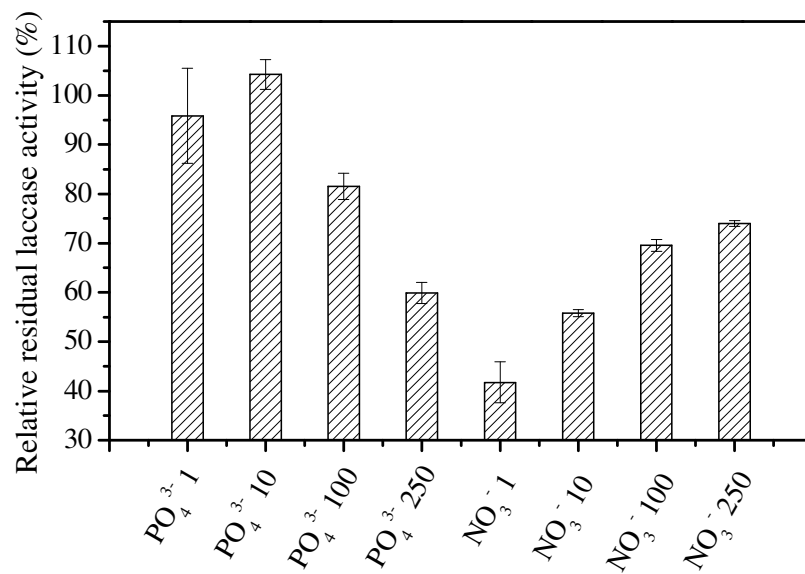


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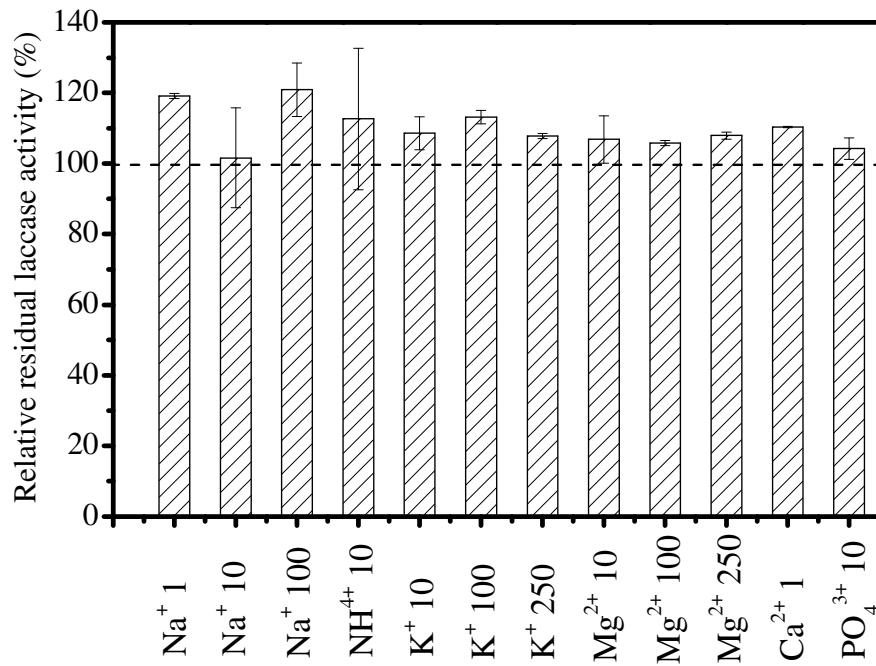


Figure 6: Relative residual laccase activity in presence of selected cations and anions showing an increase in laccase activity after incubation period. The results are normalised against the respective values in the control experiment conducted in absence of salt.

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Table 1: Selected cations and anions

Cations	Original salts	Anions	Original salts
Na^+	Na_2SO_4	NO_3^-	NaNO_3
Mg^{2+}	MgSO_4	PO_4^{3-}	Na_2PO_4
NH_4^+	$(\text{NH}_4)_2\text{SO}_4$	Cl^-	NaCl
Ca^{2+}	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	F^-	NaF
Cu^{2+}	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	I^-	KI
Zn^{2+}	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	Br^-	KBr
Mn^{2+}	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$		
K^+	K_2SO_4		
Al^{3+}	$\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$		