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

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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^+, NH_4^+, 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. Mg^{2+} 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.

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Introduction

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

Enzymatic transformation of TrOCs has recently attracted much attention as a promising eco-friendly concept (Yang et al. 2013). Laccase (Benzenediol: oxygen oxidoreductase; EC 1.10.3.2) is of interest as it only requires molecular oxygen as a co-substrate, unlike the peroxidases which require H_2O_2. Laccase catalysed oxidation mechanism includes the reduction of molecular oxygen to water and one electron oxidation of various aromatic substrates such as TrOCs (Majeau et al. 2010; Nguyen et al. 2014b; Yang et al. 2013). Laccase has been found to be abundant in many white-rot fungi. The potential of laccase for the removal of TrOCs has been investigated intensively by various researchers. Results have demonstrated that laccase can effectively degrade a range of TrOCs (Majeau et al. 2010; Nguyen et al. 2014b; Spina et al. 2015; Tran et al. 2010; Yang et al. 2013). TrOC removal by laccase is governed by factors such as pH, temperature, and physicochemical properties of TrOCs (Asif et al. 2017a). For example, optimum pH for the removal of triclosan was in the range of 5 to 5.5 (Kim & Nicell 2006b), while diclofenac was found to be removed at the highest rate under acidic conditions (pH 3 - 4.5) (Nguyen et al. 2014a). TrOC properties can also strongly influence laccase performance. The compounds which contain phenolic moiety are more amendable to laccase. On the other hand, compounds which contains functional group such as carboxylic, amide, and chloride are resistant to laccase oxidation (Asif et al. 2017b; Shi et al. 2017; Tran et al. 2010; Yang et al. 2013).

Only a few studies have provided some insight into the effect of heavy metals on the removal of dye by laccase (Murugesan et al. 2009; Rodríguez Couto et al. 2005). Murugesan et al. (2009) reported that metal ions such as Ca^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} at a concentration of 1 mM did not have impact on laccase performance. However, it is hypothesized that some ions may block or interfere with the active sites of laccase and thus decrease its activity (Asif et al. 2017b; 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 $\mu M_{(DMP)}$/ min) and negligible peroxidase activity. The

115　crude laccase preparation thus obtained was stored at 4 °C until use.

Batch test description

A strategic experimental plan was implemented in this study. Kim and Nicell (2006a), reported negligible impact of Na^+ and NH_4^+ on laccase activity. Therefore, at first, SO_4^{2-} dissolved from sodium and ammonium sulfate salts was tested to elucidate if SO_4^{2-} has low impact so that sulfate salts can be used as a source of cations. After confirming the low impact of SO_4^{2-}, a range of sulfate salts (Table 1) was used.

To test the variance in influence of the anions, tests were conducted to include PO_4^{3-} and NO_3^-, and four halides i.e., F^-, I^-, Br^- and Cl^-. The anions were matched with either Na^+ or K^+ to select the salt to be added to the test solution (Table 1).

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

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

Analytical methods

Laccase activity

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

TrOC analysis

A HPLC system (Shimadzu, Kyoto, Japan), equipped with a Supelco Drug Discovery 300×4.6 mm C-18 column (5 µm pore size) and a UV–vis detector, was used to measure the TrOC concentrations. The detection wavelength was 280 nm and the column temperature was 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 μg/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).

Results and discussion

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]

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^+, $NH4^+$, 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^+, NH_4^+, 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^+, NH_4^+, and K^+. Similar to laccase activity, the
213   change in Na^+, NH_4^+, 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^+, NH_4^+, 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 Mg^{2+} appear to be higher than that of the control.
221   Mg^{2+} 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   Mg^{2+} concentration-increase. Ca^{2+} affected laccase activity at 100 mM. However, the effect of
226   Ca^{2+} on TrOC removal was significant even at 1 mM (Figure 3). Murugesan et al. (2009)
227   observed a concentration-dependent effect of Ca^{2+} 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 Zn^{2+} on laccase and its dye
231   decolourisation capacity. Similarly, in the current study, irrespective of its concentration, Zn^{2+}
232   showed moderate inhibition of laccase activity and TrOC removal (Figure 3). Mn^{2+} 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 Mn^{2+} (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).

Impact of anions

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: $F^- > I^- > Br^- > Cl^-$. 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   <div align="center">[FIGURE 5]</div>

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   <div align="center">[FIGURE 6]</div>

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^+, NH_4^+ 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.

Disclosure of conflict of interest

335 The authors report no conflicts of interest.

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423

List of Tables

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+}	$(NH_4)_2SO_4$		Cl^-	$NaCl$
Ca^{2+}	$CaSO_4.2H_2O$		F^-	NaF
Cu^{2+}	$CuSO_4.5H_2O$		I^-	KI
Zn^{2+}	$ZnSO_4.7H_2O$		Br^-	KBr
Mn^{2+}	$MnSO_4.4H_2O$			
K^+	K_2SO_4			
Al^{3+}	$Al_2(SO_4)_3.16H_2O$			

List of Figures

Figure 1: Impact of sulfate (SO_4^{2-}) on laccase stability and removal of BPA and DCF. Error bars present the standard deviation of duplicate samples. The dotted line indicates the initial laccase activity.

Figure 2: Effect of different cations on laccase activity and BPA and DCF removal. The cations were dissolved from sulfate salts at a concentration of 250 mM. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

Figure 3: Effect of cation concentrations on laccase activity and BPA and DCF removal. The cations were dissolved from sulfate salts. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

Figure 4: Effect of halides on laccase activity and BPA and DCF removal. The anions were obtained from sodium and potassium salts. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

Figure 5: Effect of PO_4^{3-} and NO_3^{-} on laccase activity. The anions were dissolved from sodium salts. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

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.
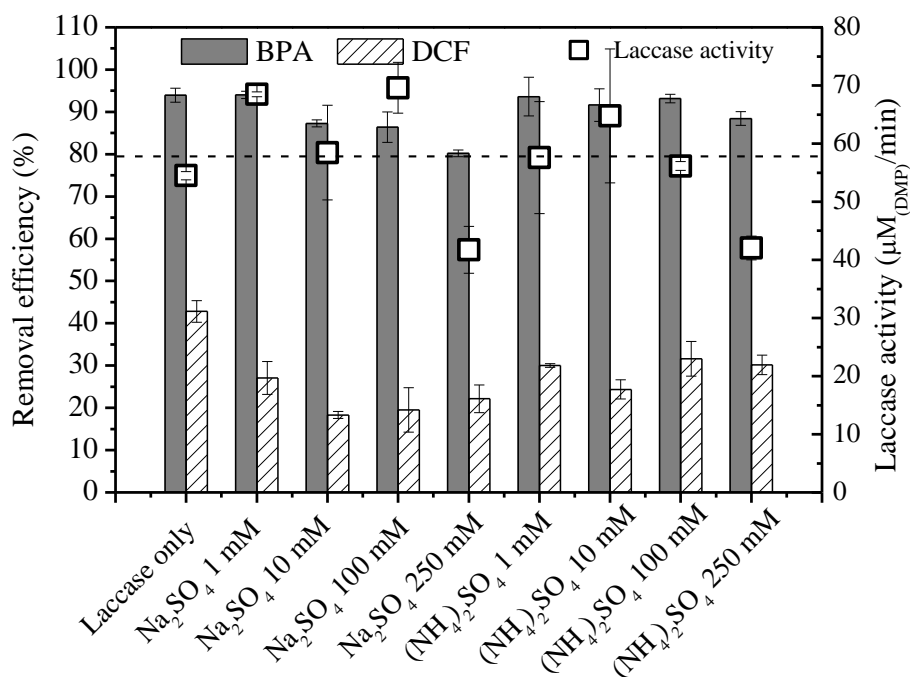
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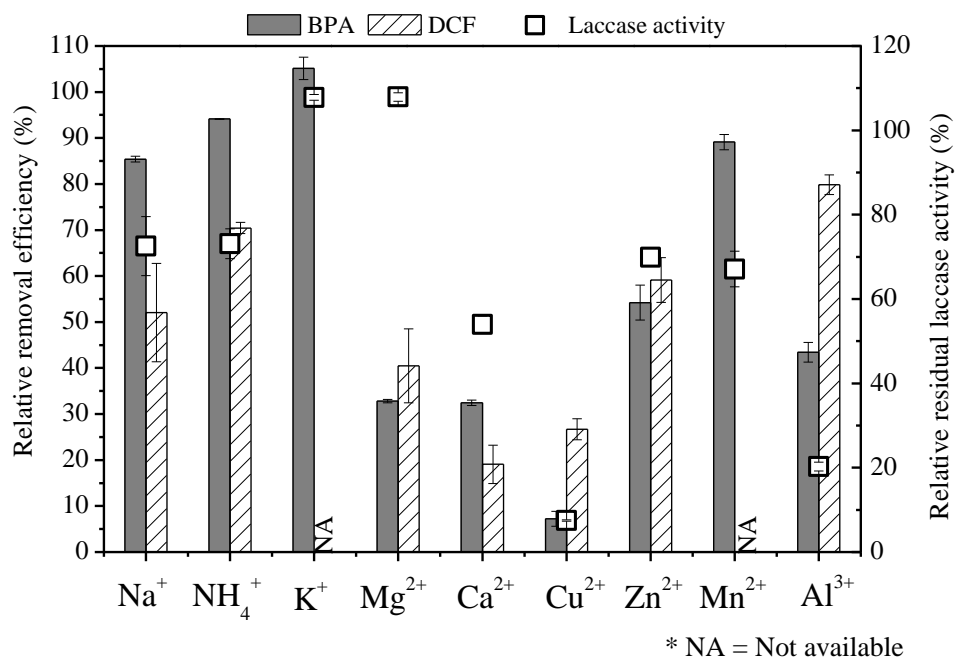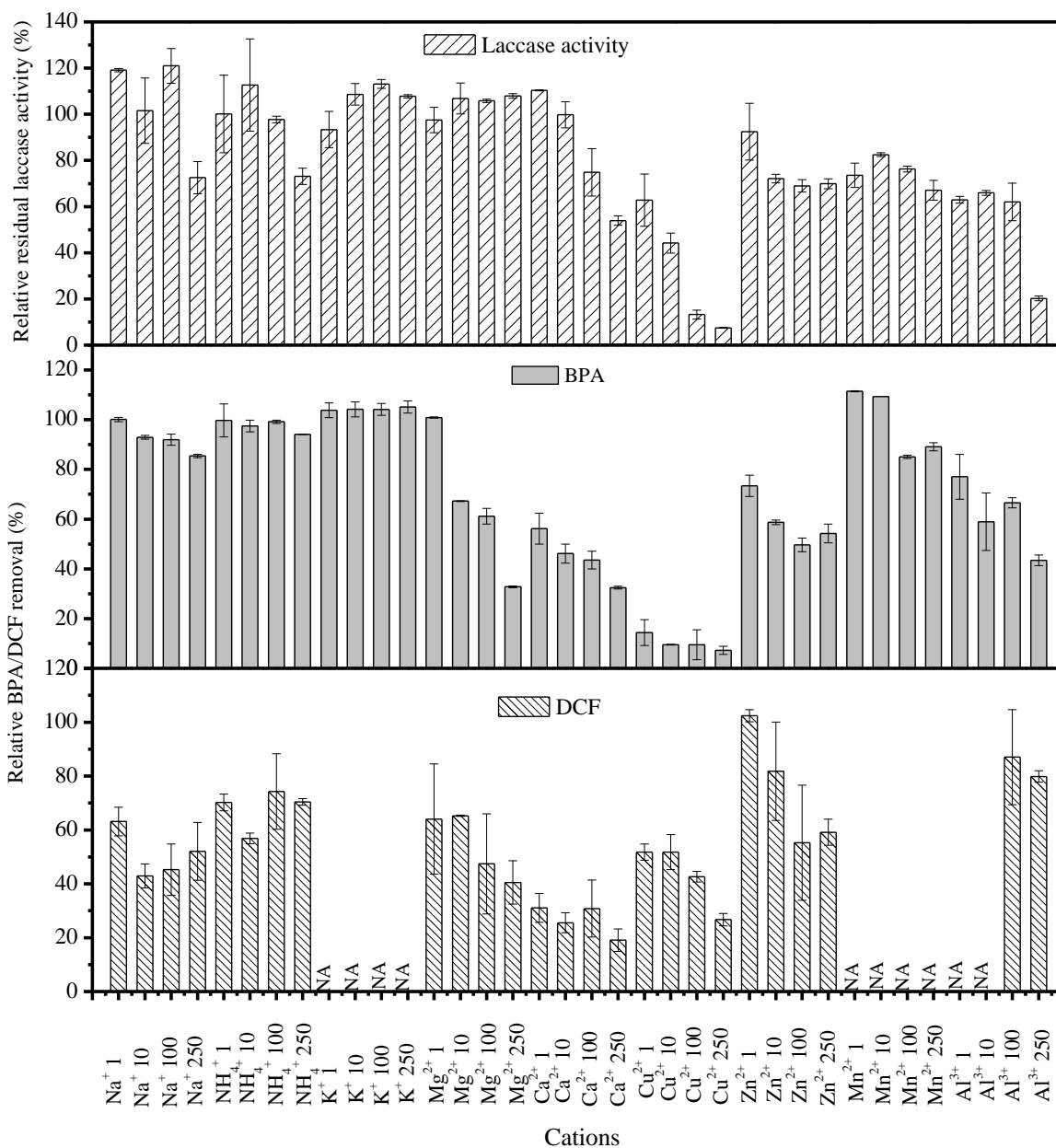
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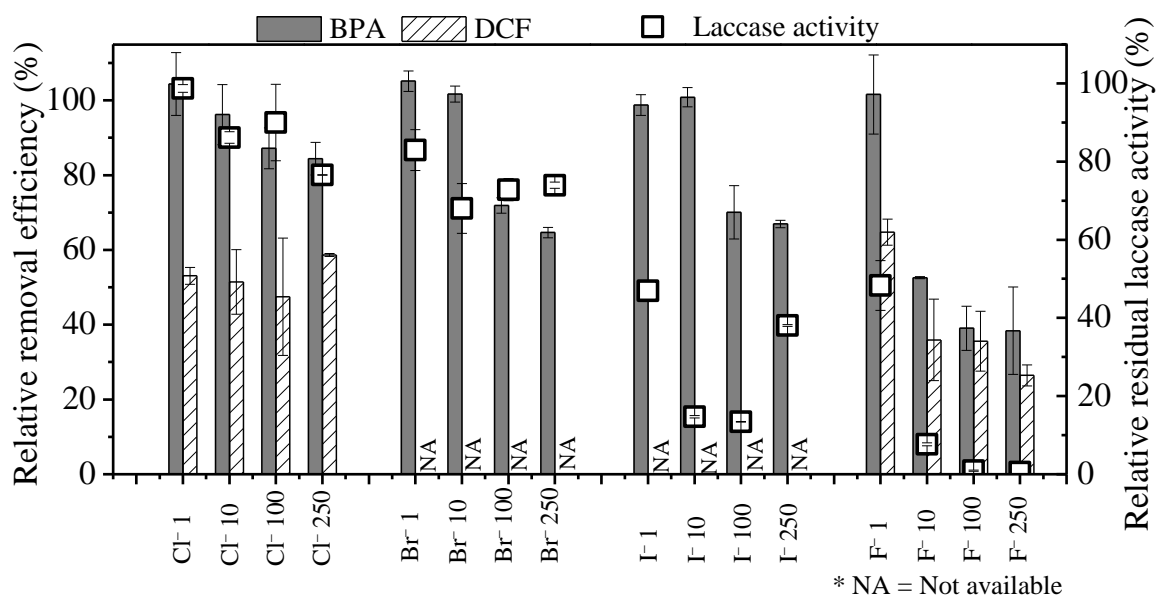
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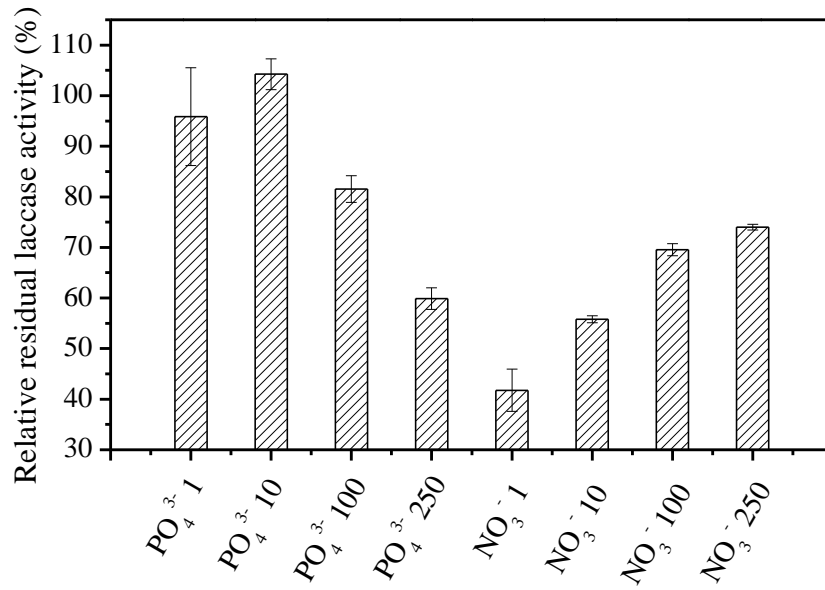
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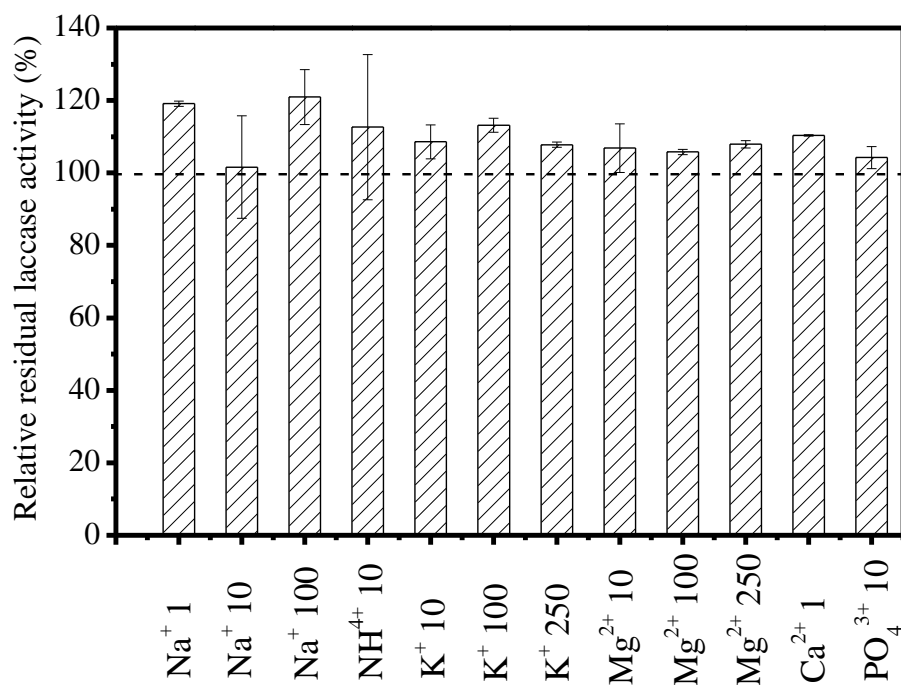
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