Supplementary Information for the manuscript: Pharmaceutical Pollution in Marine Waters and Benthic Flora of the Southern Australian Coastline

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Analysis of pharmaceutical concentration in wastewater treatment plant effluent, ocean surface water samples, and benthic flora

P-values included in the manuscript are highlighted. Packages used: ggplot2, ggpubr, dplyr, FSA

Read wastewater treatment plant data from CSV file:

```
wwtp = read.csv("wwtp.csv",check.names = FALSE)
head(wwtp)
```

```
##        sample  conc        pharma      site
## 1 Breamlea 1 892.8 Carbamazepine Breamlea
## 2 Breamlea 2 881.9 Carbamazepine Breamlea
## 3 Breamlea 3 806.6 Carbamazepine Breamlea
## 4    Lorne 1 425.5 Carbamazepine    Lorne
## 5    Lorne 2 453.8 Carbamazepine    Lorne
## 6    Lorne 3 440.3 Carbamazepine    Lorne
```

Visualise wastewater treatment plant data as a barplot

```
ggbarplot(wwtp, x = "pharma", y = "conc", fill="site",
 add = "mean_se", position=position_dodge())
```
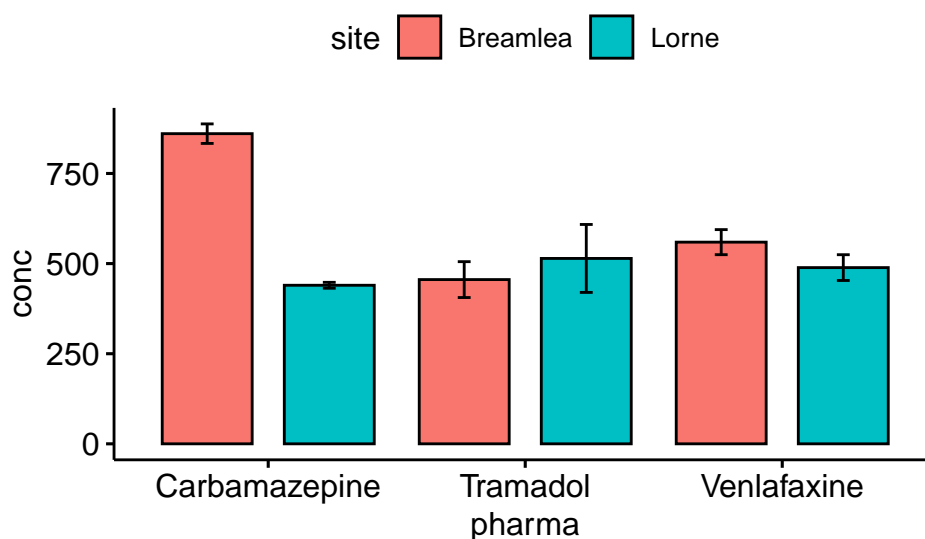
Test for differences in WWTP concentration for carbamazepine

```r
wwtp_carb<-wwtp %>% filter(pharma=="Carbamazepine")
wilcox.test(conc ~ site, data = wwtp_carb,exact=FALSE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  conc by site
## W = 9, p-value = 0.08086
## alternative hypothesis: true location shift is not equal to 0
```

```r
with(wwtp_carb, shapiro.test(conc[site == "Breamlea"]))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  conc[site == "Breamlea"]
## W = 0.84313, p-value = 0.2223
```

```r
with(wwtp_carb, shapiro.test(conc[site == "Lorne"]))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  conc[site == "Lorne"]
## W = 0.9993, p-value = 0.9494
```

```r
t.test(conc ~ site, data = wwtp_carb,var.equal = TRUE)
```

```
##
##  Two Sample t-test
##
## data:  conc by site
## t = 14.858, df = 4, p-value = 0.0001195
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##   341.9783 499.1551
## sample estimates:
## mean in group Breamlea    mean in group Lorne
##               860.4333               439.8667
```

Test for differences in WWTP concentration for tramadol

```r
wwtp_tram<-wwtp %>% filter(pharma=="Tramadol")
wilcox.test(conc ~ site, data = wwtp_tram,exact=FALSE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  conc by site
## W = 4, p-value = 1
## alternative hypothesis: true location shift is not equal to 0
```

```r
with(wwtp_tram, shapiro.test(conc[site == "Breamlea"]))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  conc[site == "Breamlea"]
## W = 0.92572, p-value = 0.4728
```

```r
with(wwtp_tram, shapiro.test(conc[site == "Lorne"]))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  conc[site == "Lorne"]
## W = 0.78365, p-value = 0.07605
```

```r
t.test(conc ~ site, data = wwtp_tram,var.equal = TRUE)
```

```
##
##  Two Sample t-test
##
## data:  conc by site
## t = -0.55076, df = 4, p-value = 0.6111
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -354.6144  237.2144
## sample estimates:
## mean in group Breamlea    mean in group Lorne
##                  455.6                  514.3
```

Test for differences in WWTP concentration for venlafaxine

```r
wwtp_ven<-wwtp %>% filter(pharma=="Venlafaxine")
wilcox.test(conc ~ site, data = wwtp_ven,exact=FALSE)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  conc by site
## W = 8, p-value = 0.1904
## alternative hypothesis: true location shift is not equal to 0
```

```r
with(wwtp_ven, shapiro.test(conc[site == "Breamlea"]))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  conc[site == "Breamlea"]
## W = 0.79724, p-value = 0.1079
```

```r
with(wwtp_ven, shapiro.test(conc[site == "Lorne"]))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  conc[site == "Lorne"]
## W = 0.90131, p-value = 0.3897
```

```r
t.test(conc ~ site, data = wwtp_ven,var.equal = TRUE)
```

```
##
##  Two Sample t-test
##
## data:  conc by site
## t = 1.4165, df = 4, p-value = 0.2296
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -67.81669 209.08335
## sample estimates:
```

```
## mean in group Breamlea    mean in group Lorne
##             559.5000                 488.8667
```

Read marine surface water data from CSV file:

```
marine_surface = read.csv("marine_surface.csv",check.names = FALSE)
head(marine_surface)
```

```
##   sample   conc       pharma        site
## 1  TRBW1 0.1506 Carbamazepine  Breamlea
## 2  TRBW2 0.2414 Carbamazepine  Breamlea
## 3  TRBW3 0.5029 Carbamazepine  Breamlea
## 4  UQBW1 0.9865 Carbamazepine Urquharts
## 5  UQBW2 0.4644 Carbamazepine Urquharts
## 6  UQBW3 0.1484 Carbamazepine Urquharts
```

Visualise wastewater treatment plant data as a barplot

```
ggbarplot(marine_surface, x = "pharma", y = "conc", fill="site",
 add = "mean_se", position=position_dodge())
```
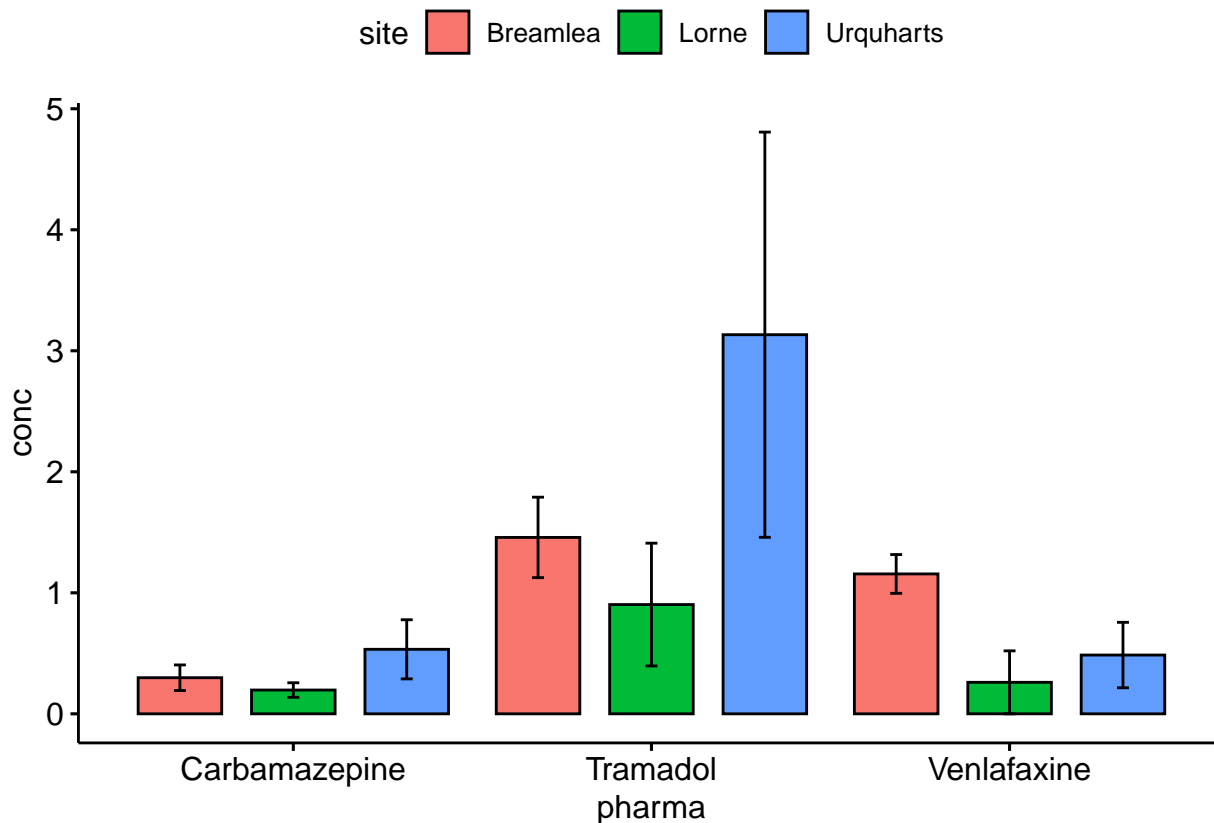

Test for difference in surface water concentration for each pharmaceutical

```
carb<-marine_surface %>% filter(pharma=="Carbamazepine")
kruskal.test(conc ~ site, data = carb)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  conc by site
```

```
## Kruskal-Wallis chi-squared = 1.1556, df = 2, p-value
## = 0.5611
```

```
dunnTest(conc ~ site,
         data=carb,
         method="bonferroni")
```

```
##            Comparison          Z   P.unadj      P.adj
## 1     Breamlea - Lorne  0.7453560 0.4560565 1.0000000
## 2 Breamlea - Urquharts -0.2981424 0.7655945 1.0000000
## 3    Lorne - Urquharts -1.0434984 0.2967175 0.8901526
```

```
tram<-marine_surface %>% filter(pharma=="Tramadol")
kruskal.test(conc ~ site, data = tram)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  conc by site
## Kruskal-Wallis chi-squared = 1.098, df = 2, p-value =
## 0.5775
```

```
dunnTest(conc ~ site,
         data=tram,
         method="bonferroni")
```

```
##            Comparison          Z   P.unadj      P.adj
## 1     Breamlea - Lorne  0.5239368 0.6003225 1.0000000
## 2 Breamlea - Urquharts -0.5239368 0.6003225 1.0000000
## 3    Lorne - Urquharts -1.0478737 0.2946968 0.8840905
```

```
ven<-marine_surface %>% filter(pharma=="Venlafaxine")
kruskal.test(conc ~ site, data = ven)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  conc by site
## Kruskal-Wallis chi-squared = 4.7816, df = 2, p-value
## = 0.09156
```

```
dunnTest(conc ~ site,
         data=ven,
         method="bonferroni")
```

```
##            Comparison          Z    P.unadj      P.adj
## 1     Breamlea - Lorne  2.1226745 0.03378114 0.1013434
## 2 Breamlea - Urquharts  1.5161961 0.12946978 0.3884093
## 3    Lorne - Urquharts -0.6064784 0.54419710 1.0000000
```

Read marine flora data from CSV file:

```
marine = read.csv("marine.csv",check.names = FALSE)
head(marine)
```

```
##   sample       pharma site   species      conc
## 1      1 Carbamazepine Turd Hormosira 0.000000
## 2      2 Carbamazepine Turd Hormosira 3.094928
## 3      3 Carbamazepine Turd Hormosira 0.000000
## 4      4 Carbamazepine Turd    Codium 2.998006
## 5      5 Carbamazepine Turd    Codium 0.000000
## 6      6 Carbamazepine Turd    Codium 0.000000
```

Visualise wastewater treatment plant data as a barplot

```
ggbarplot(marine, x = "pharma", y = "conc", fill="site",
 add = "mean_se", position=position_dodge())
```
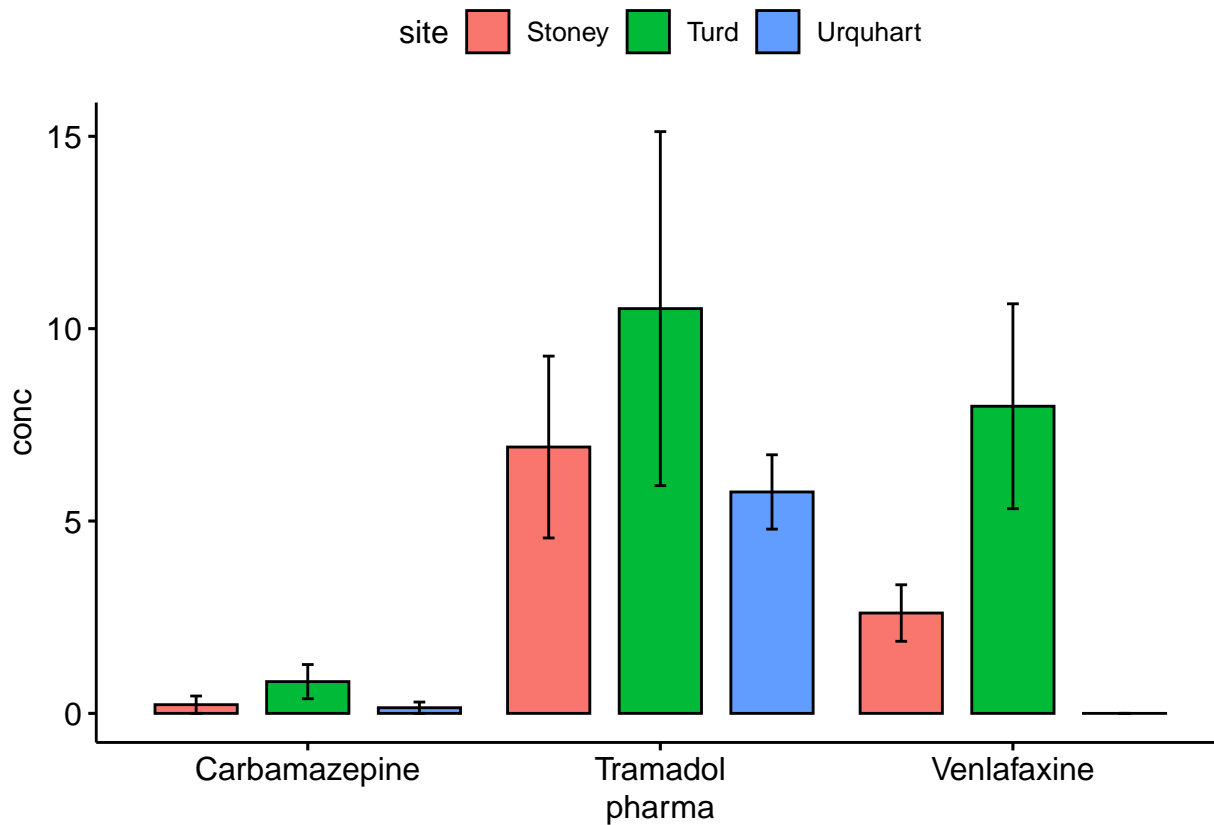

Test for differences in carbamazepine concentration in Hormosira

```
hormosira<-marine %>% filter(species=="Hormosira")
horm_carb<-hormosira %>% filter(pharma=="Carbamazepine")
kruskal.test(conc ~ site, data = horm_carb)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  conc by site
## Kruskal-Wallis chi-squared = 3, df = 2, p-value =
## 0.2231
```

```
dunnTest(conc ~ site,
         data=horm_carb,
         method="bonferroni")
```

```
##         Comparison         Z   P.unadj     P.adj
## 1      Stoney - Turd -1.632993 0.1024704 0.3074113
## 2 Stoney - Urquhart  0.000000 1.0000000 1.0000000
## 3   Turd - Urquhart  1.414214 0.1572992 0.4718976
```

Test for differences in tramadol concentration in Hormosira

```r
hormosira<-marine %>% filter(species=="Hormosira")
horm_tram<-hormosira %>% filter(pharma=="Tramadol")
kruskal.test(conc ~ site, data = horm_tram)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  conc by site
## Kruskal-Wallis chi-squared = 6.8702, df = 2, p-value
## = 0.03222
```

```r
dunnTest(conc ~ site,
         data=horm_tram,
         method="bonferroni")
```

```
##           Comparison         Z    P.unadj      P.adj
## 1     Stoney - Turd -2.6194653 0.008806773 0.02642032
## 2 Stoney - Urquhart -0.7858396 0.431961470 1.00000000
## 3   Turd - Urquhart  1.5879664 0.112293927 0.33688178
```

Test for differences in venlafaxine concentration in Hormosira

```r
hormosira<-marine %>% filter(species=="Hormosira")
horm_ven<-hormosira %>% filter(pharma=="Venlafaxine")
kruskal.test(conc ~ site, data = horm_ven)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  conc by site
## Kruskal-Wallis chi-squared = 8.188, df = 2, p-value =
## 0.01667
```

```r
dunnTest(conc ~ site,
         data=horm_ven,
         method="bonferroni")
```

```
##           Comparison        Z    P.unadj      P.adj
## 1     Stoney - Turd -2.033553 0.041996671 0.12599001
## 2 Stoney - Urquhart  1.220132 0.222414885 0.66724466
## 3   Turd - Urquhart  2.817774 0.004835785 0.01450736
```