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Supplementary Materials for

Application of effect-based methods (EBMs) in a river basin: a preliminary study in Central Italy

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This PDF file includes:
Detailed description of the statistical analysis performed with the aid of the SAS® software and of the related boxplots

Fish Embryo Toxicity (FET) Test

We performed non-parametric tests for our statistical analysis. We considered the ranks (for N data, the highest value is N and the lowest is 1). The Kruskal-Wallis Test allow the comparison among the sampling sites in the two different years (2018 and 2019) and the different time pattern showed by the Delta (values of 2019 – values of 2018). Four variables were considered: lethal 2018, lethal 2019, sublethal 2018 and sublethal 2019. The statistical analysis was not significant for all the considered variables (significance is expressed as Pr > Ch-square and it has to be equal to ≤0.05. The boxplots show the data. A boxplot was built for each variable. Histograms are associated to the groups and they report a bar indicating the median. The highest and lowest values are reported over and under the bar and they are expressed as scores.

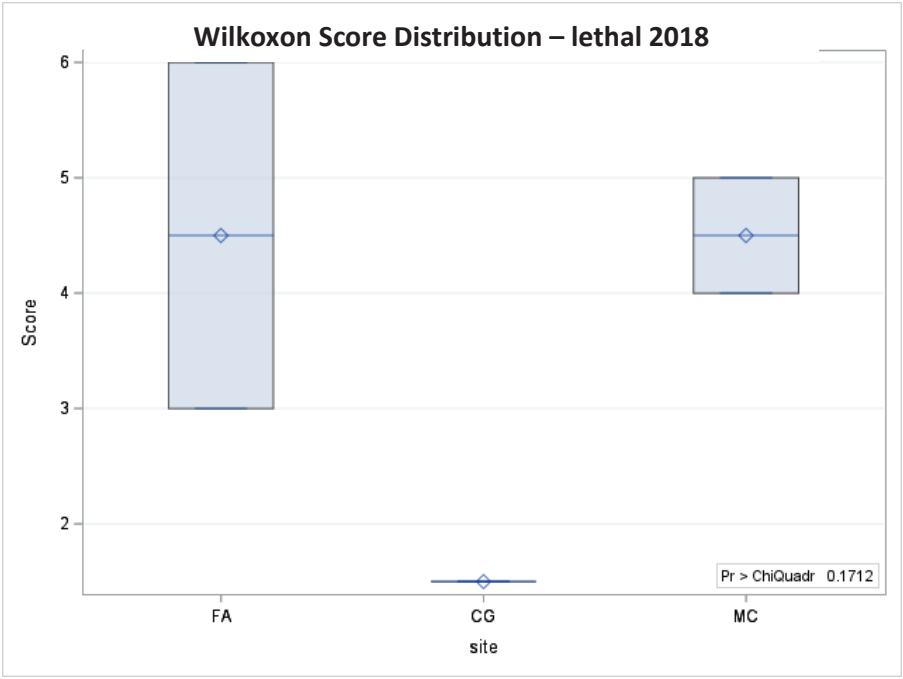
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *lethal2018* classified by the variable *site*.

site	N	Sum of the scores	Expected under H0	Standard deviation under H0	Mean score
FA	2	9.0	7.0	2.129163	4.50
CG	2	3.0	7.0	2.129163	1.50
MC	2	9.0	7.0	2.129163	4.50

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	3.5294
DF	2
Pr > Chi-square	0.1712



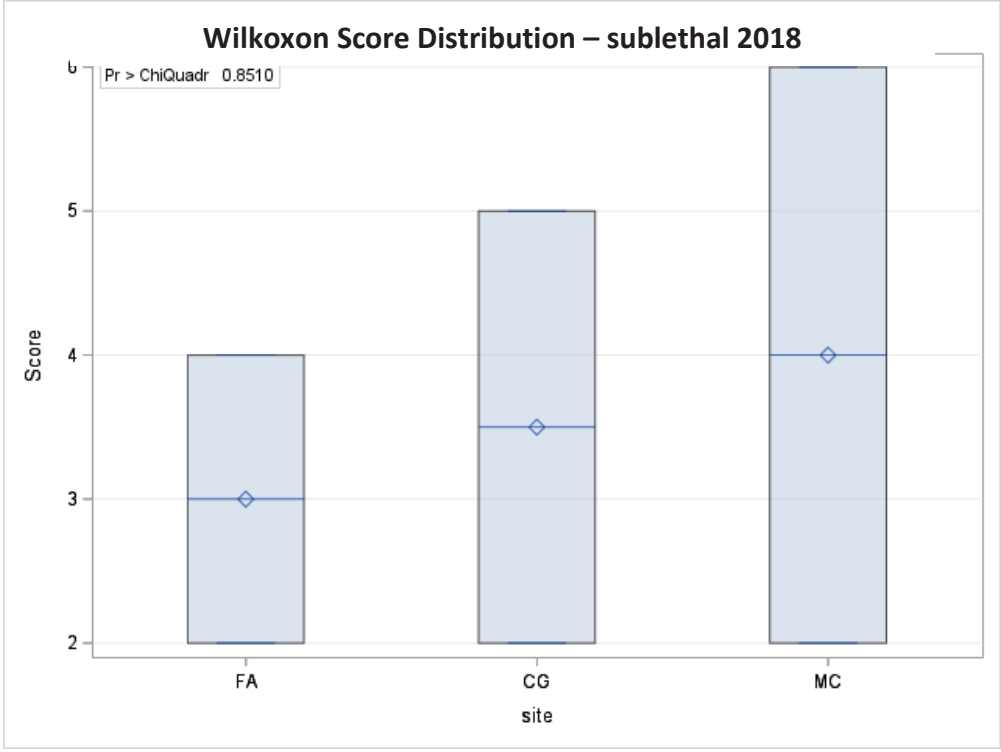
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *sublethal2018* classified by the variable *site*.

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	2	6.0	7.0	2.033060	3.00
CG	2	7.0	7.0	2.033060	3.50
MC	2	8.0	7.0	2.033060	4.00

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	0.3226
DF	2
Pr > Chi-square	0.8510



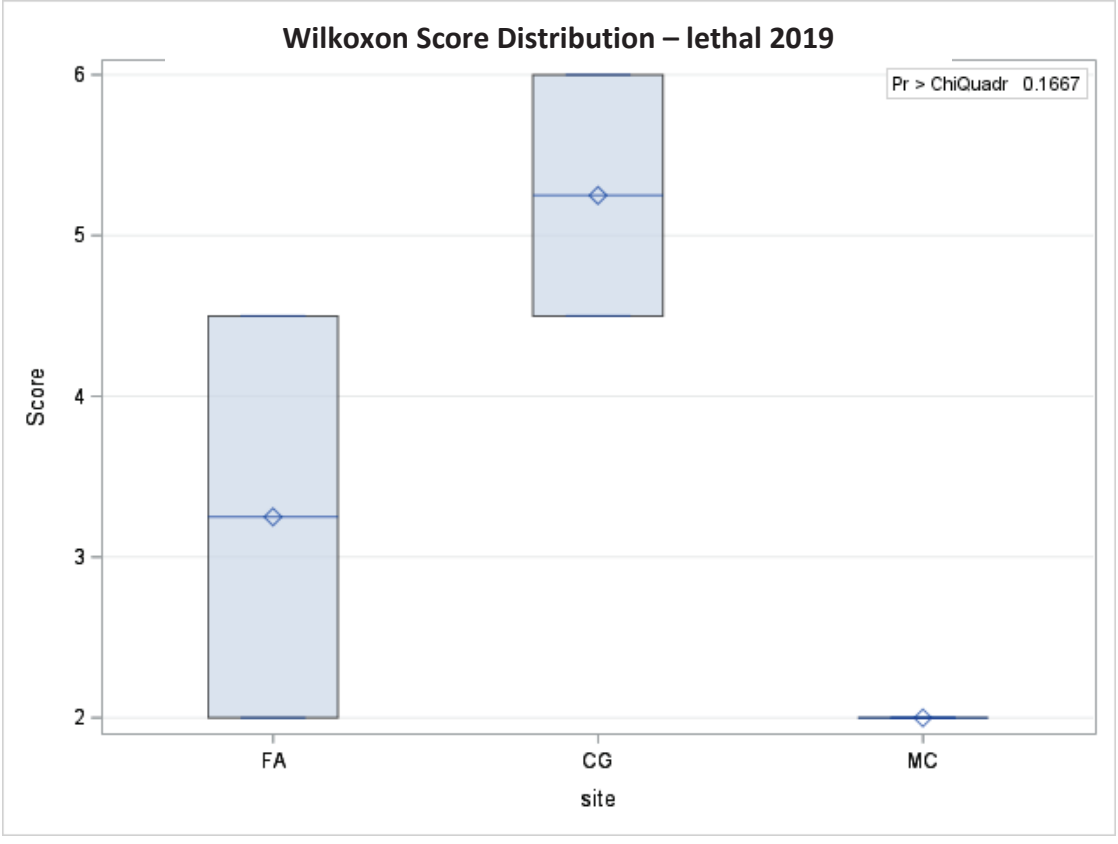
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *lethal 2019* classified by the variable *site*

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	2	6.50	7.0	2.0	3.250
CG	2	10.50	7.0	2.0	5.250
MC	2	4.00	7.0	2.0	2.000

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	3.5833
DF	2
Pr > Chi-square	0.1667



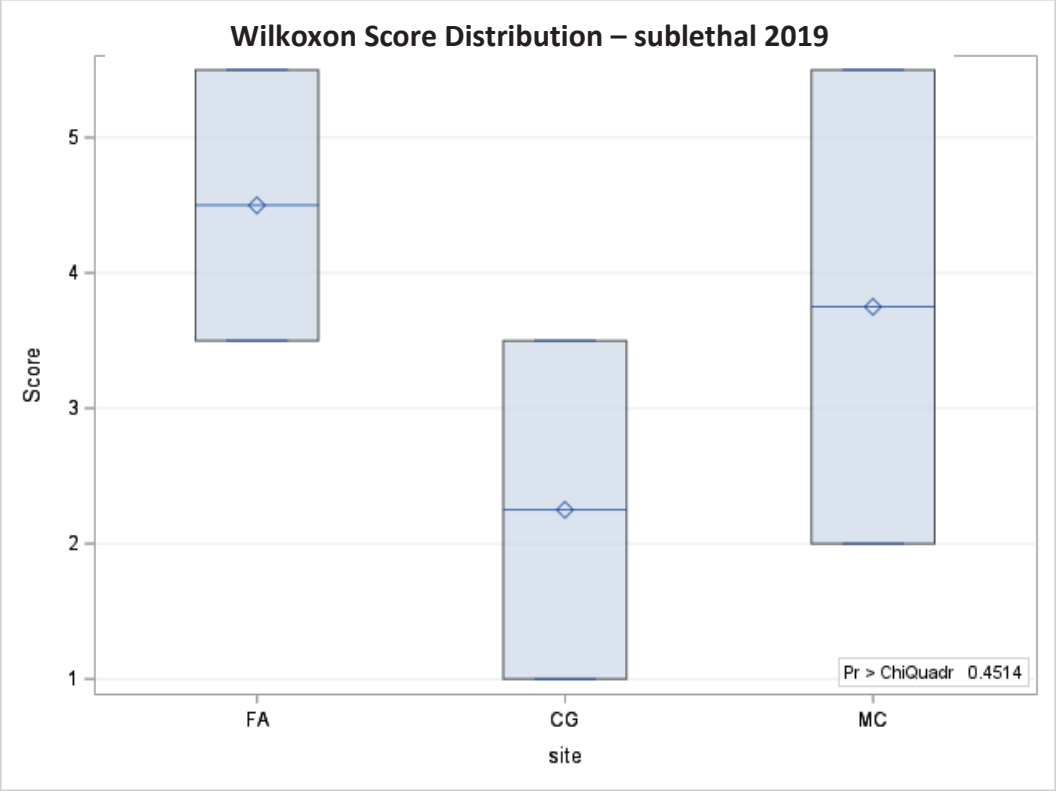
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *sublethal 2019* classified by the variable *site*

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	2	9.00	7.0	2.097618	4.500
CG	2	4.50	7.0	2.097618	2.250
MC	2	7.50	7.0	2.097618	3.750

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	1.5909
DF	2
Pr > Chi-square	0.4514



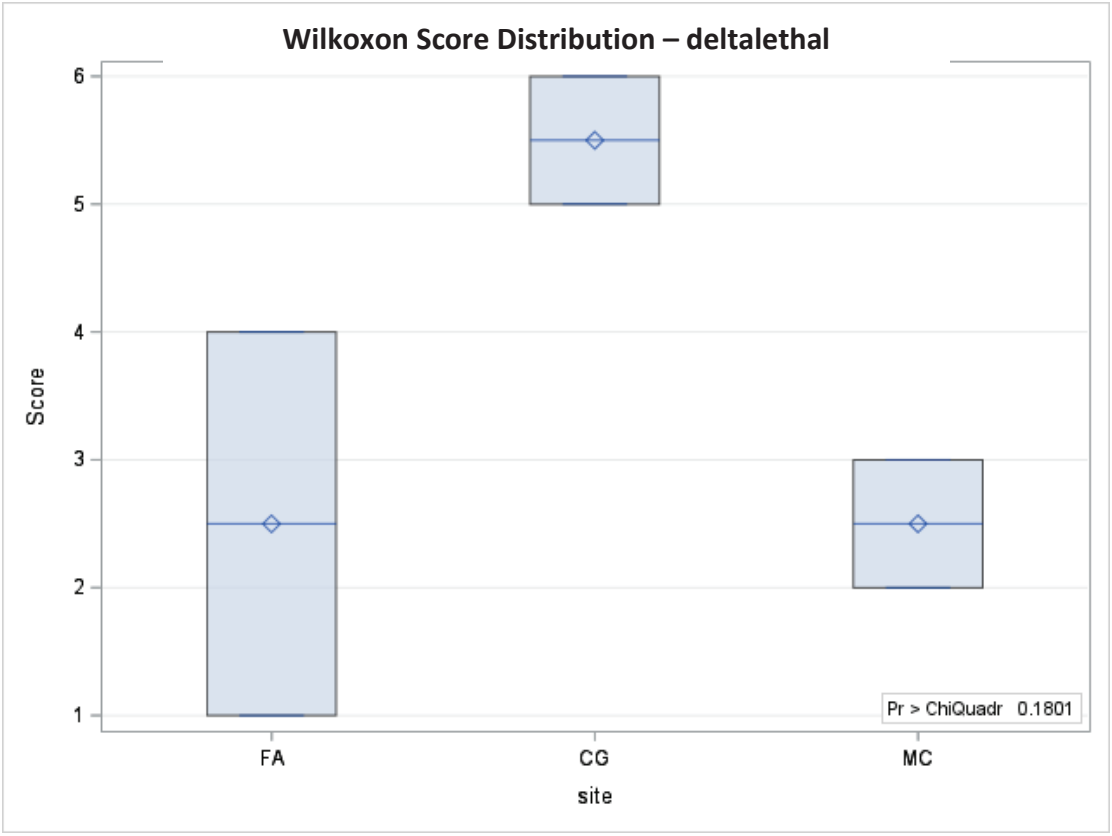
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *deltalethal* classified by the variable *site*

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	2	5.0	7.0	2.160247	2.50
CG	2	11.0	7.0	2.160247	5.50
MC	2	5.0	7.0	2.160247	2.50

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	3.4286
DF	2
Pr > Chi-square	0.1801



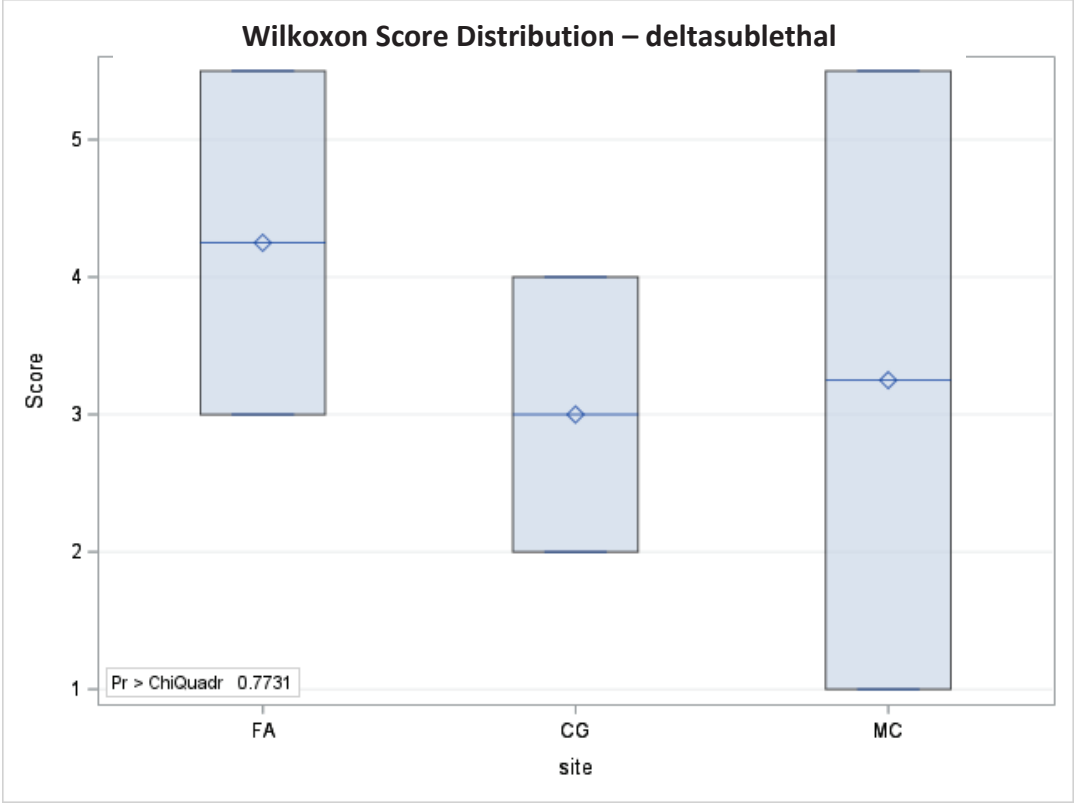
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *deltasublethal* classified by the variable *site*

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	2	8.50	7.0	2.129163	4.250
CG	2	6.00	7.0	2.129163	3.000
MC	2	6.50	7.0	2.129163	3.250

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	0.5147
DF	2
Pr > Chi-square	0.7731



Daphnia magna Immobilisation Assay

We performed non-parametric tests for our statistical analysis. We considered the ranks (for N data, the highest value is N and the lowest is 1). The Kruskal-Wallis Test allow the comparison among the sampling sites in the two different years (2018 and 2019) and the different time pattern showed by the Delta (values of 2019 – values of 2018). The statistical analysis was significant (yellow mark) for the variables *y2018* and *delta* (significance is expressed as Pr > Ch-square and it has to be equal to ≤0.05). However, these test only offer an indicative value because the samples are too few. The boxplots show the data. A boxplot was built for each variable. Histograms are associated to the groups and they report a bar indicating the median. The highest and lowest values are reported over and under the bar and they are expressed as scores.

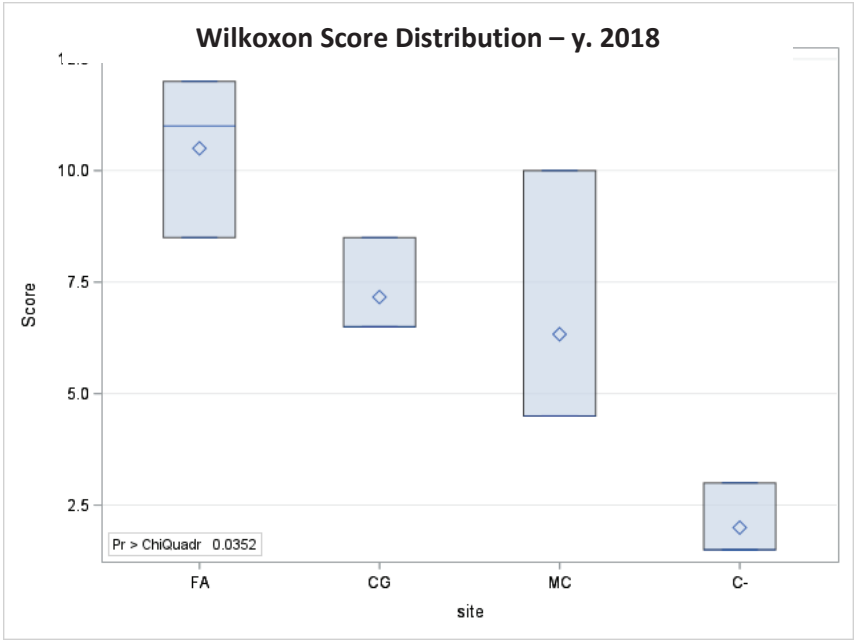
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *a2018* classified by the variable *site*

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	3	31.50	19.50	5.370373	10.500000
CG	3	21.50	19.50	5.370373	7.166667
MC	3	19.00	19.50	5.370373	6.333333
C-	3	6.00	19.50	5.370373	2.000000

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	8.5946
DF	3
Pr > Chi-square	0.0352



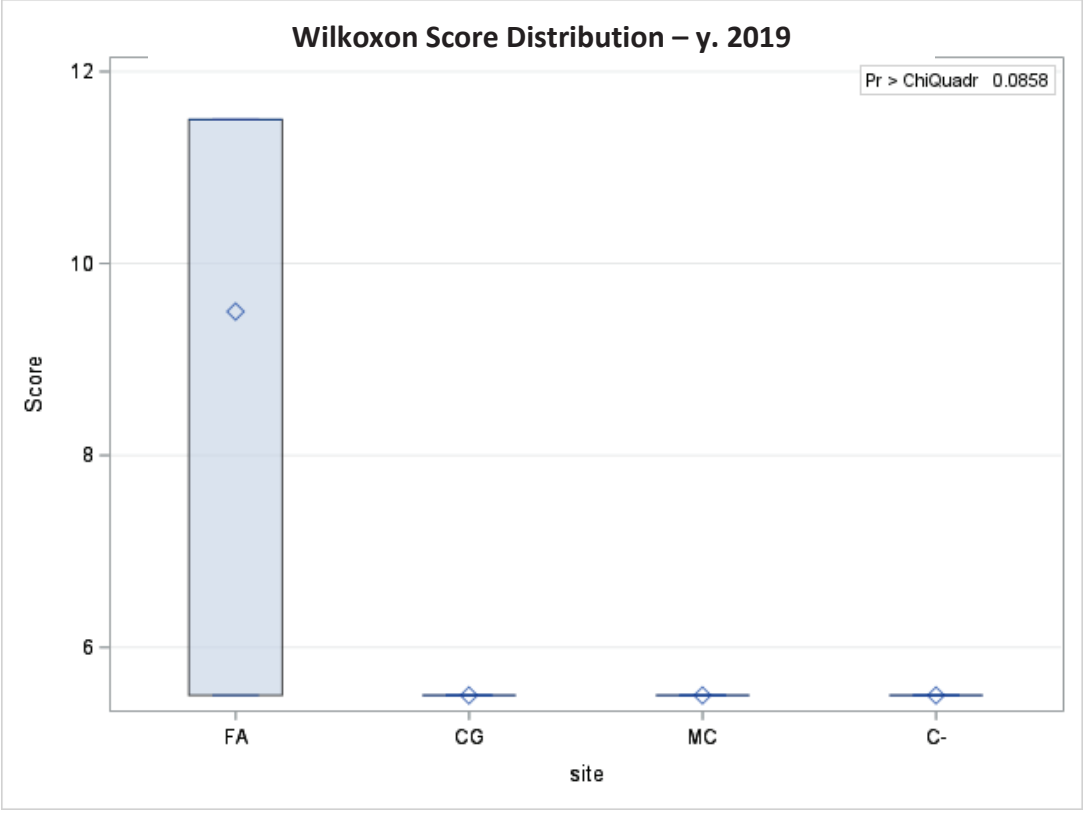
The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *a2019* classified by the variable *site*

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	3	28.50	19.50	3.503245	9.50
CG	3	16.50	19.50	3.503245	5.50
MC	3	16.50	19.50	3.503245	5.50
C-	3	16.50	19.50	3.503245	5.50

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	6.6000
DF	3
Pr > Chi-square	0.0858



The procedure NPAR1WAY

Wilcoxon score (sums of the ranks) for the variable *delta* classified by the variable *site*

site	N	Sum of the core	Expected under H0	Standard deviation under H0	Mean score
FA	3	7.50	19.50	5.370373	2.500000
CG	3	17.50	19.50	5.370373	5.833333
MC	3	20.00	19.50	5.370373	6.666667
C-	3	33.00	19.50	5.370373	11.000000

Mean scores were used for the equivalent values.

Kruskal-Wallis Test	
Chi-square	8.5946
DF	3
Pr > Chi-square	0.0352