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# Trends of the hydrometeorological variables in the wider area of the Zagreb aquifer

Original scientific paper

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## Abstract

The aim of this paper was to estimate trends of hydrometeorological variables in different time scales in order to see whether it will generate an indication of the impact of climate change in the wider research area. Trends were estimated on a monthly and yearly basis for precipitation, air temperature, evapotranspiration and maximum water available for infiltration at meteorological station Pleso, and for Bregana River water levels at hydrological station Koretići. Both locations are important because they can show patterns that could be characteristic for the wider Zagreb area, and are associated with Zagreb aquifer recharge. Linear regression and t-test were used for trend estimation. Monthly analysis showed ascending trends for air temperature and evapotranspiration, especially in the summer months. Descending trends for Bregana River water levels were observed when minimum and average values were considered, while statistically significant ascending trend for maximum water levels was observed only for February. Yearly trends showed similar patterns. Moreover, it was shown that evaluation of trends only on a yearly basis can sometimes hide important information. All results suggest that more dry periods should be expected, while floods could be more frequent in February. Bigger difference between high and low waters, i.e. extremes, was observed, but the results were not statistically significant. Trends showed that smaller infiltration from precipitation and reduced inflow from Bregana River to Sava River will probably result with the smaller recharge to the Zagreb aquifer, what can potentially be result of a climate change.

**Keywords:** trend; hydrometeorological variable; Zagreb aquifer; linear regression

## 1. Introduction

Trend evaluation of basic hydrometeorological variables, i.e. flow, river water level, precipitation, air temperature and evapotranspiration, is very important because it can show how human impacts and/or some natural events can influence on the availability of water in different areas. A lot of shallow alluvial aquifers are directly connected with rivers where the estimation of river water level trends can directly show which kind of groundwater level trends can be expected and estimate how will groundwater seasonal water reserves change in the future. The same situation is with observation and definition of trends. Some variables have direct influence on effective infiltration, i.e. the water that participates in the replenishment of seasonal groundwater reserves through the water percolation from the unsaturated zone into the aquifers. Also, changes in runoff, frequency and regime of flows and precipitation can present the evidence of human impact through the increase of greenhouse gases which can affect the water cycle. This can be seen in the form of flooding, droughts and heavy precipitation (Groisman et al., 2005; Barnett et al., 2008; Murphy et al., 2013; Pavlić et al., 2017). Furthermore, EU Water Framework Directive (2000/60/EC; WFD) and Groundwater Directive (2006/118/EC; GD) have shown that trends have to be calculated for a groundwater body or a group of bodies, where appropriate, in the procedure related with the definition of chemical and quantitative status and risk assessment. Although trends estimation can be done with different methods, the nonparametric Mann-Kendall test (Mann, 1945; Kendall, 1975) is maybe the most often used method in the trend estimation of hydroclimatic and water quality data series (Gebremedhin et al., 2016; Lutz et al., 2016; Pavlić et al., 2017; Diamantini et al., 2018). However, other methods like linear regression, piecewise regression and logistic regression can be used in the estimation of trends of hydrological and water quality time series (Grath et al., 2001; Yue et al., 2002; Kovač et al., 2018).

In this paper, the focus is not on the application of different statistical methods, but on the data aggregation, which was done on the monthly and yearly basis. It was tested whether the data aggregation and evaluation of data in different time intervals can generate different conclusions. Also, one of the aims was to see if there was an impact of a climate change in the wider research area. For that purpose, linear regression was used to estimate trends on meteorological variables at meteorological station Pleso in the area of Zagreb aquifer, while trends on hydrological variables were estimated on the data from hydrological station Koretići, which is located in the upstream of the Bregana River. Evaluation

of trends for both locations is very important, first because it can be used for the estimation of maximum values available for the effective infiltration which participates in the recharge of seasonal groundwater reserves of the Zagreb aquifer, and other due to possible flooding of inhabited areas of the Bregana River. Previous statistical studies of meteorological variables in the research area were focused on the analysis of daily, monthly and yearly values of the precipitation at meteorological station Zagreb-Grič. In that research it was concluded that precipitation trend is not the consequence of the climate change or urbanization (Bonacci and Roje-Bonacci, 2019). Ivezić et al. (2019) have done statistical analysis on the hydrological stations of the Bregana River. They investigated hydrological extremes due to more often flooding in the year 2005, 2014 and 2015. In that research trends were estimated for yearly maximum river flows. Results showed ascending trends of maximum flows, especially after year 2000. Evaluation of trends on a monthly and yearly basis for evapotranspiration and maximum water available for infiltration have not been studied before in the research area, as well as trends related to the difference between hydrological extremes.

In Figure 1 location of meteorological station Pleso and hydrological station Koretići is shown. Both locations are located in the northwestern part of the Croatia. Meteorological station Pleso is one of the main meteorological stations in the wider area of the City of Zagreb and is located in the southeastern part of the Zagreb aquifer, which is designated as part of Country's strategic water reserves. Hydrological station Koretići is the most upstream station of the Bregana River which in extreme hydrological conditions floods areas downstream and in the end flows into the Sava River which presents the main source of recharge for the Zagreb aquifer.

Figure 1 was made in ArcMap 10.1., while geocoded terrain (orthophoto) image was obtained from geoportal of Croatian Geodetic Administration. All other calculations and figures were made with Microsoft® Excel and Power Point.

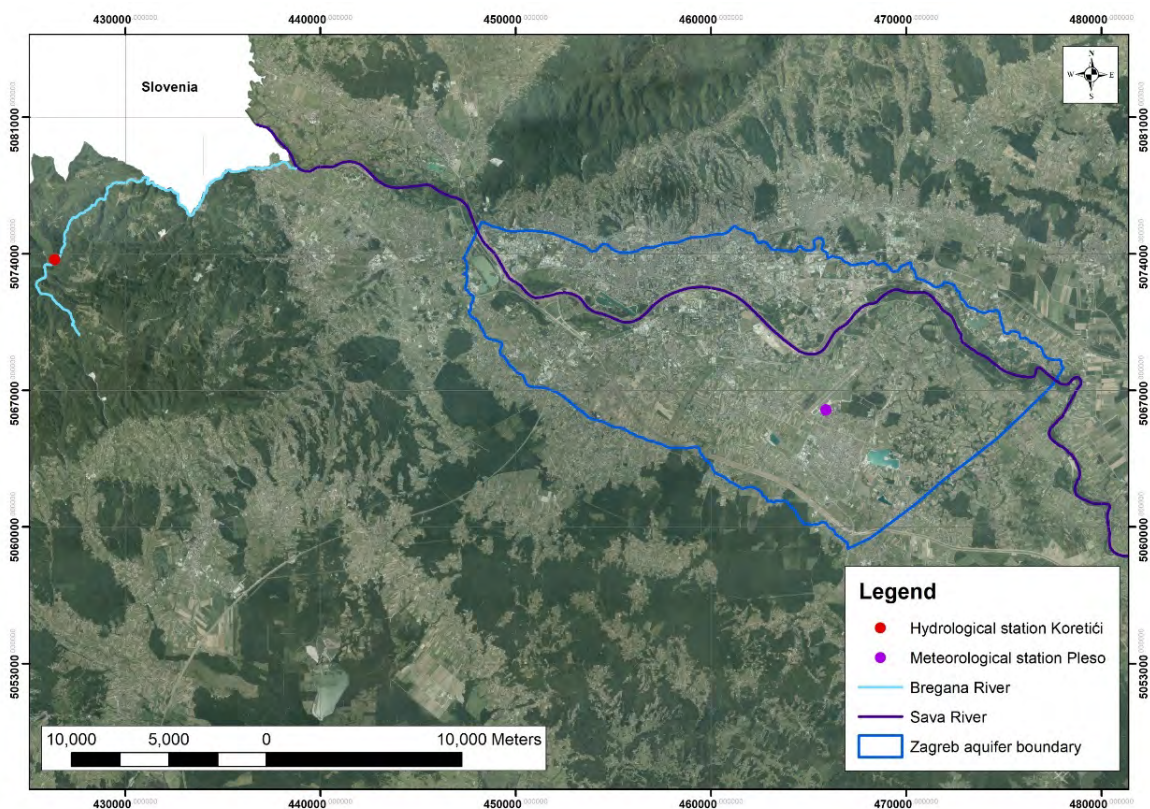


Figure 1: Location of meteorological station Pleso and hydrological station Koretići

## 2. Data and Methods

Meteorological and hydrological data for this research was given by Croatian Meteorological and Hydrological Service. Meteorological data consisted from daily precipitation and air temperature data for the meteorological station Pleso located in the southeastern part of the Zagreb aquifer. Daily data was aggregated (average values were calculated) on a monthly and yearly time period and were used for the calculation of evapotranspiration and maximum available water for infiltration into the Zagreb aquifer, and associated trends on monthly and yearly basis. Time period from 1981 till the end of 2019 was used. Data from September to December in year 1991 were not available which resulted in the exclusion of that year in the trend estimation on a yearly basis. Trends were estimated with linear regression using least squares approach, while t-test was used to calculate p-values and test statistical significance ( $\alpha=0.05$ ).

All monthly and yearly average data are presented through the histograms shown in **Figures 2 to 13**. For bin estimation **Scott (1979)** equation has been used:

$$W=3.49 \cdot \sigma \cdot N^{1/3} \quad (1)$$

where  $\sigma$  present standard deviation, while  $N$  presents number of used data.

In **Figures 2 to 6** histograms for monthly and yearly precipitation and air temperature (in °C) is presented. From these variables yearly evapotranspiration was calculated according to **Turc's formula (1953)**:

$$E_T = \frac{P}{\sqrt{0.9 + \frac{P^2}{L^2}}} \quad (2)$$

where:

$P$  – yearly precipitation (mm);

$L = 300 + 25T + 0.05T^3$ ;

$T$  – average yearly air temperature (°C).

Since the data related to monthly average air temperatures and precipitation can be calculated, for the calculation of corrected evapotranspiration air temperatures were corrected according to the equation:

$$T_p = \frac{\sum(P_i T_i)}{\sum P_i} \quad (3)$$

where:

$P_i$  – monthly precipitation (mm);

$L = 300 + 25T_p + 0.05T_p^3$ ;

$T_i$  – average monthly air temperature (°C).

Precipitation values were reduced for the value of evapotranspiration and corrected evapotranspiration to get the estimation of maximum water available for infiltration. Yearly trends were calculated for both evapotranspiration and corrected values, while estimation of yearly trends for maximum water available for infiltration was also done with evapotranspiration and corrected evapotranspiration. It can be seen that in the study area yearly precipitation values mostly vary from 700 to 1200 mm, while average air temperature varies mostly from 10 to 12 °C.

Hydrological data consisted from river water levels (cm) for the station Koretići from the year 1980 till year 2017. Daily data was aggregated to monthly and yearly basis, while minimum, maximum and average values of river water levels were used for the trend estimation (histograms are shown at **Figures 7 to 13**). For the year 1980, only data from September to December were available. Additionally, trends for difference between extremes, i.e. difference between maximum and minimum values, were calculated. Maximum water levels at station Koretići can be more expected in the May and September and minimum in August. Yearly maximum water levels are mostly between 45 and 88 cm, minimum water levels between 19 to 26 cm, while average values vary mostly between 24 and 33 cm.



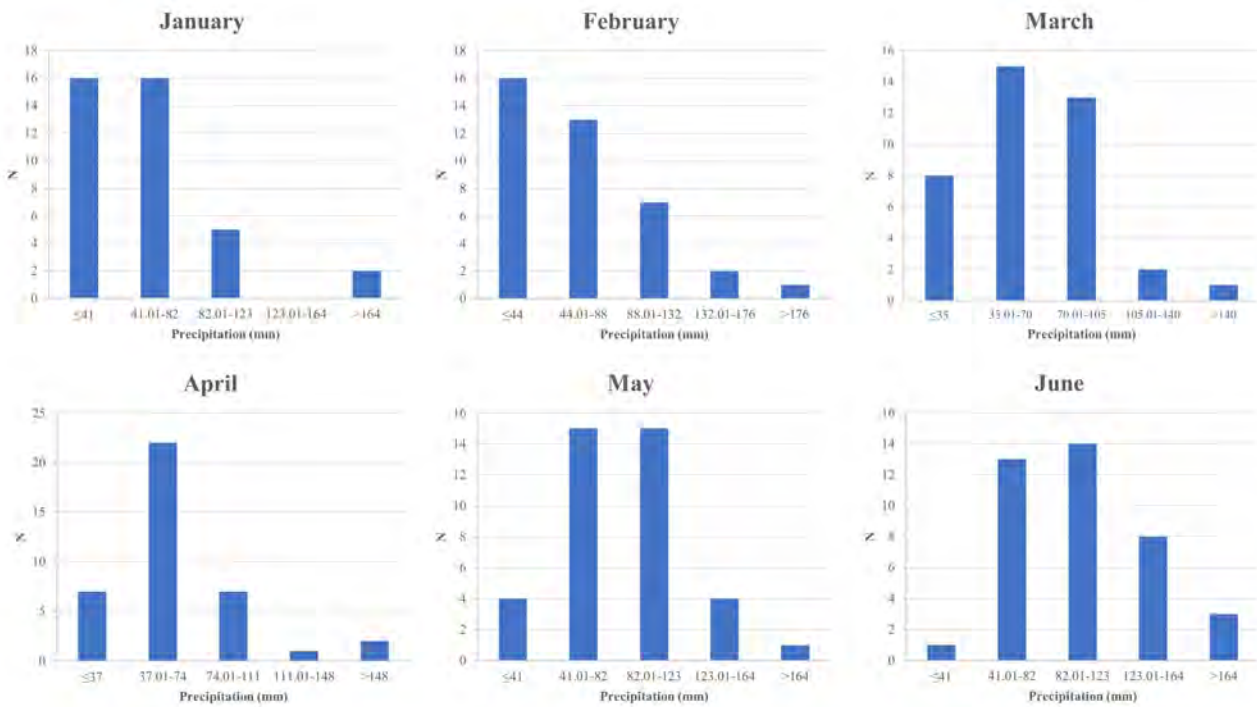


Figure 2: Histograms for average values of precipitation from January to June at meteorological station Pleso

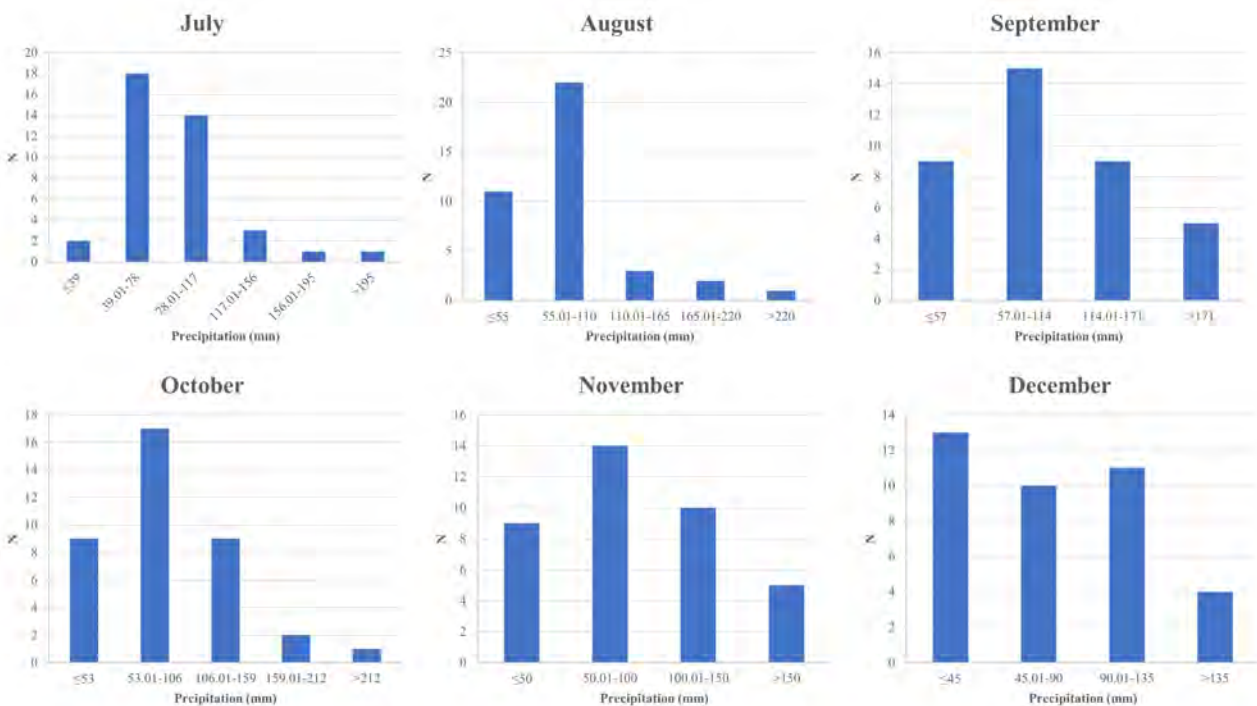


Figure 3: Histograms for average values of precipitation from July to December at meteorological station Pleso

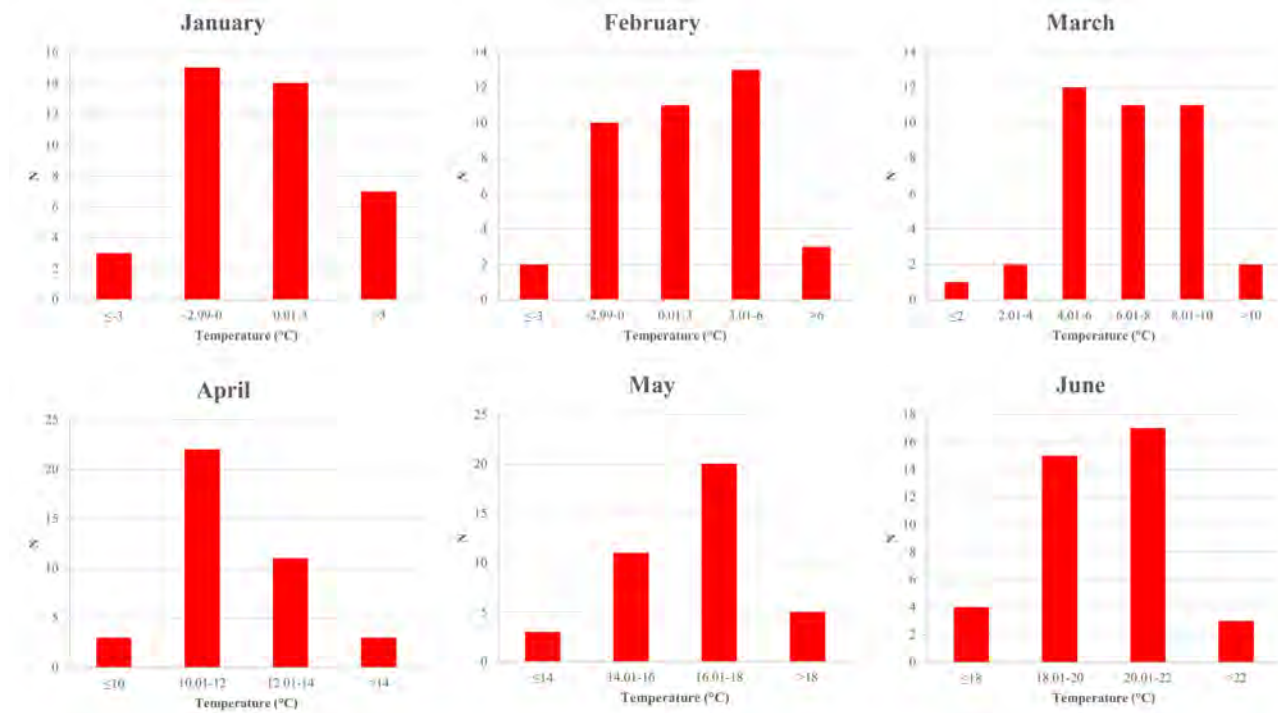


Figure 4: Histograms for average values of air temperature from January to June at meteorological station Pleso

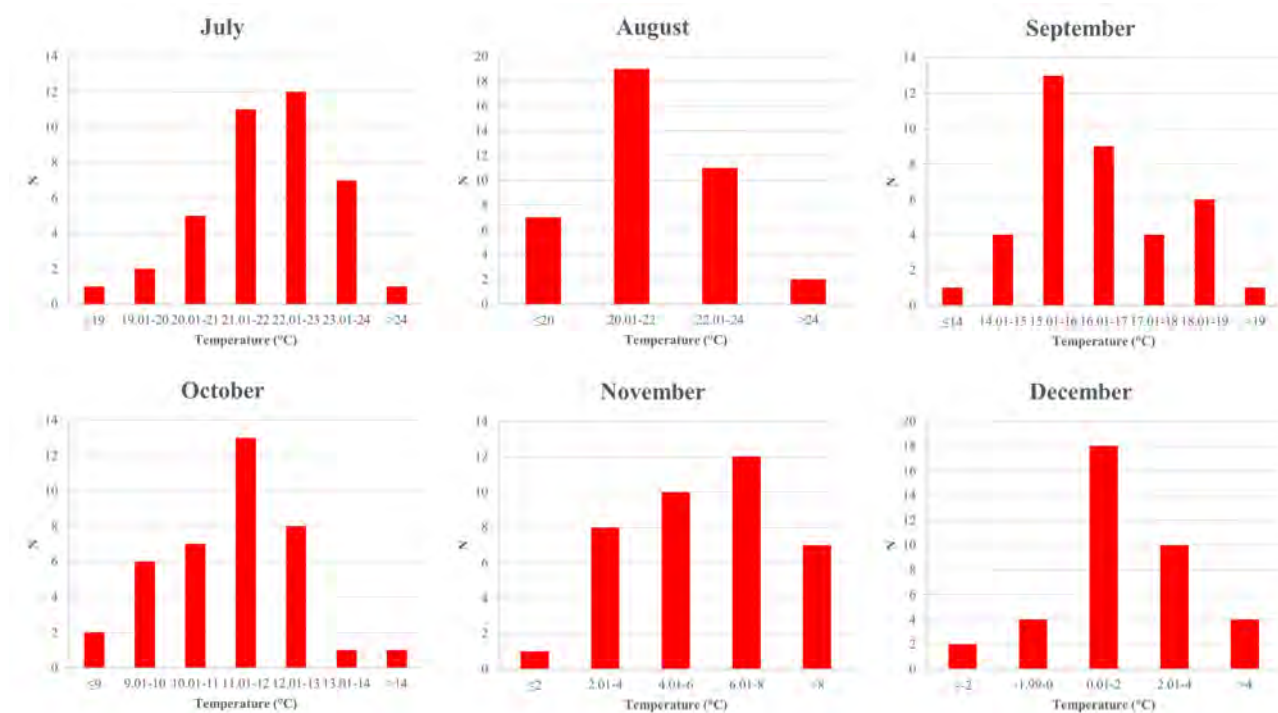


Figure 5: Histograms for average values of air temperature from July to December at meteorological station Pleso

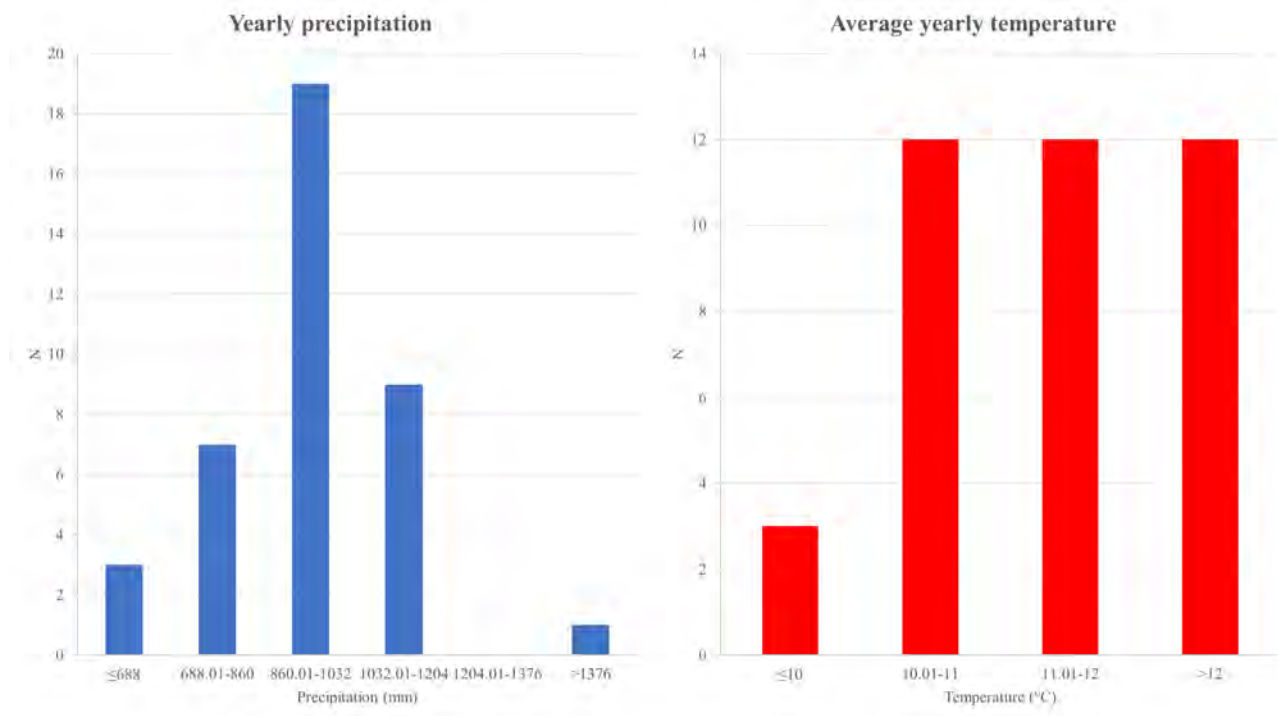


Figure 6: Histograms for average yearly values of precipitation and air temperature at meteorological station Pleso

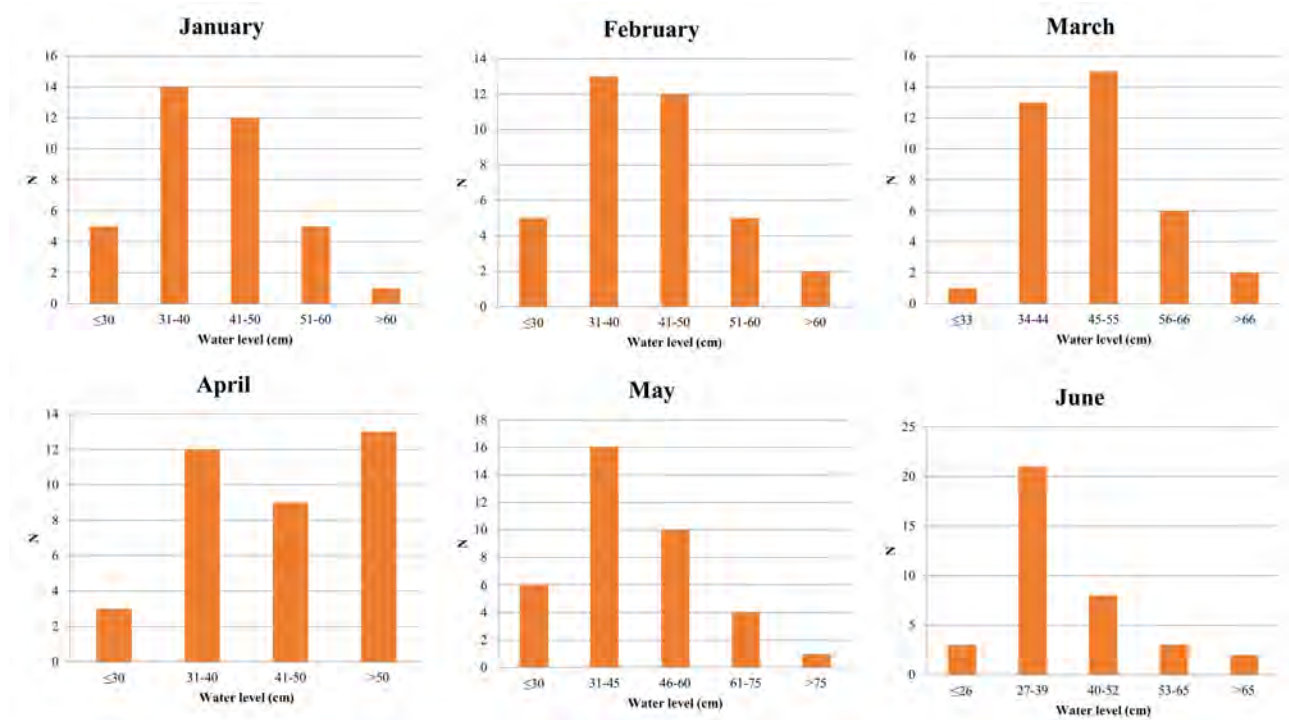


Figure 7: Histograms for maximum water levels from January to June at hydrological station Koretići

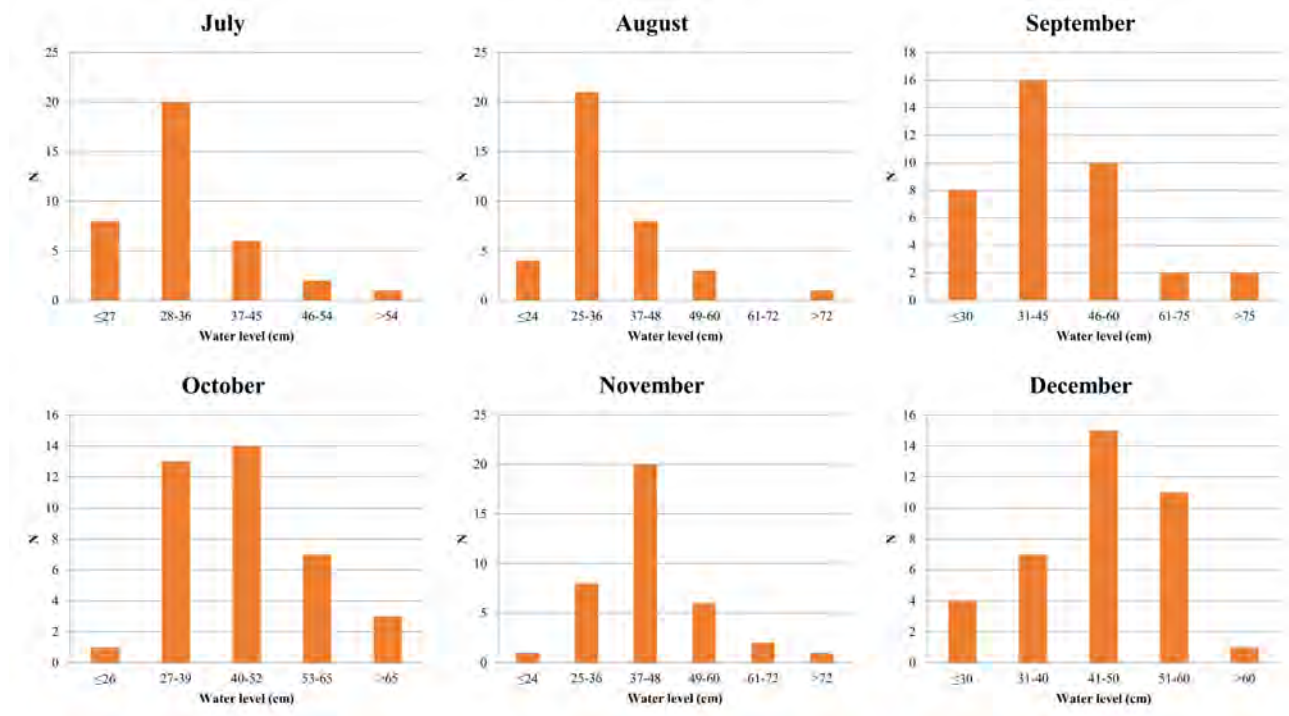


Figure 8: Histograms for maximum water levels from July to December at hydrological station Koretići

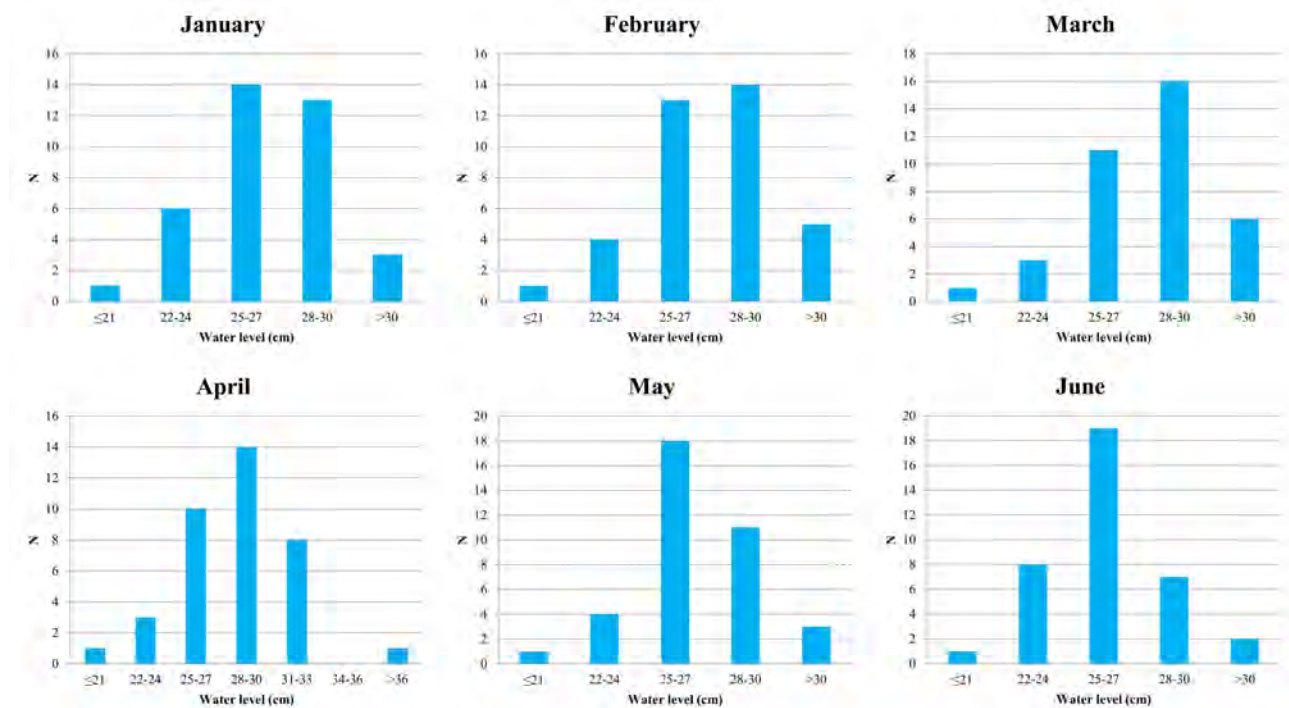


Figure 9: Histograms for minimum water levels from January to June at hydrological station Koretići



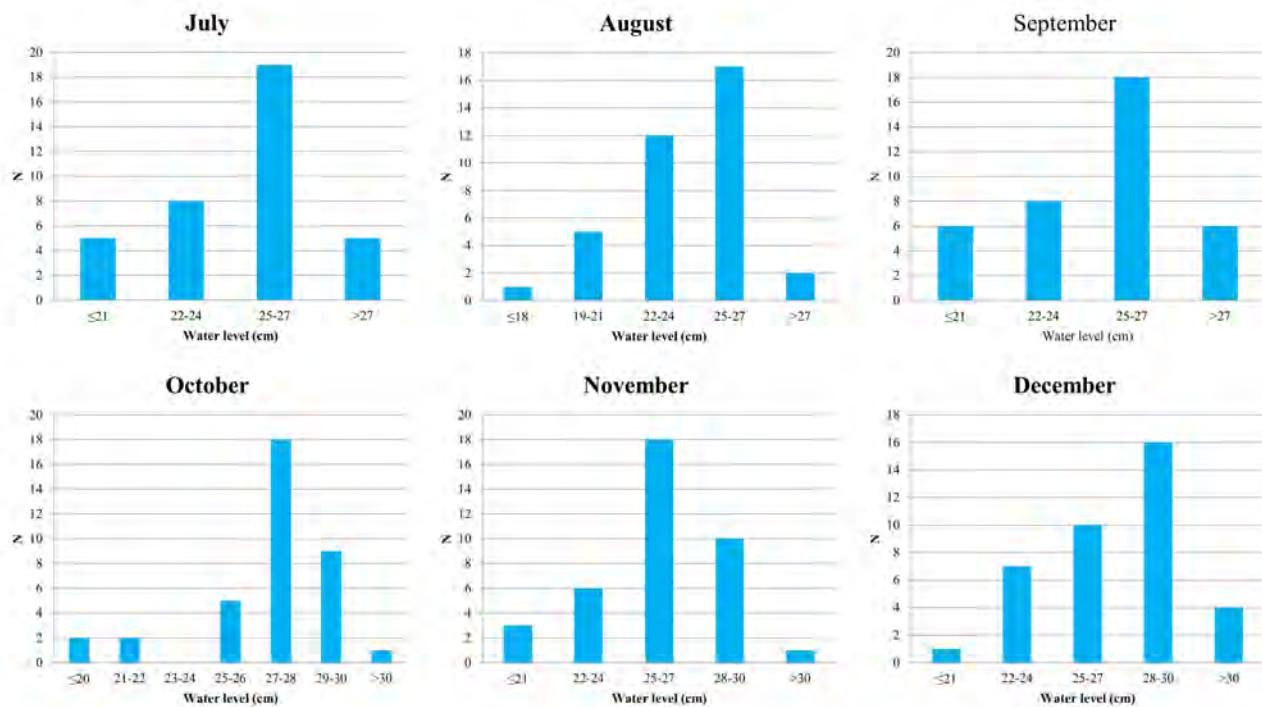


Figure 10: Histograms for minimum water levels from July to December at hydrological station Koretići

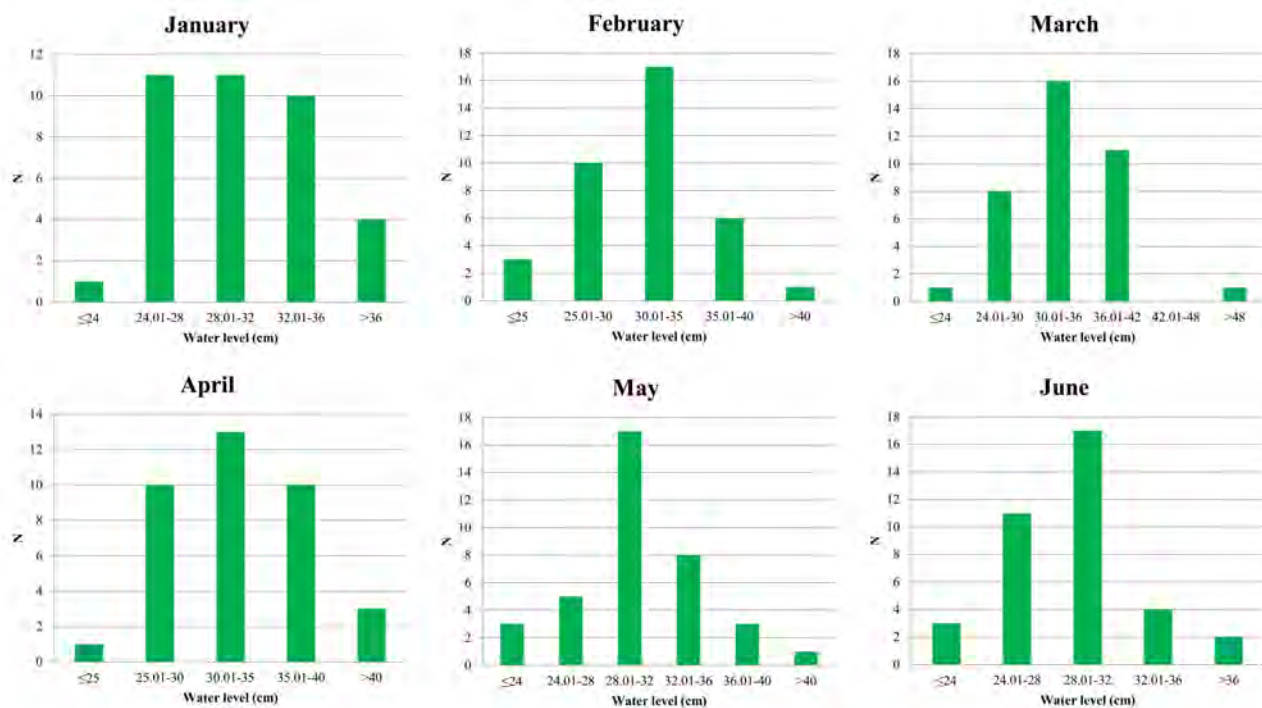


Figure 11: Histograms for average water levels from January to June at hydrological station Koretići

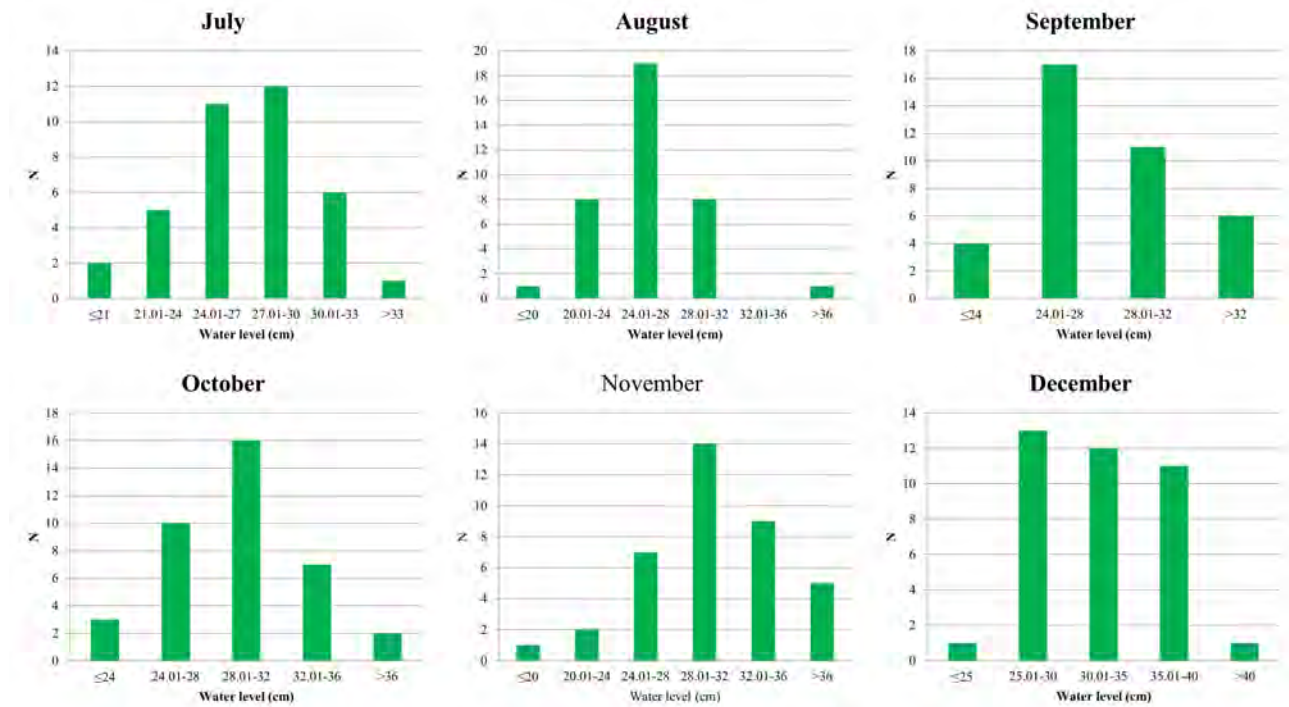


Figure 12: Histograms for average water levels from July to December at hydrological station Koretići

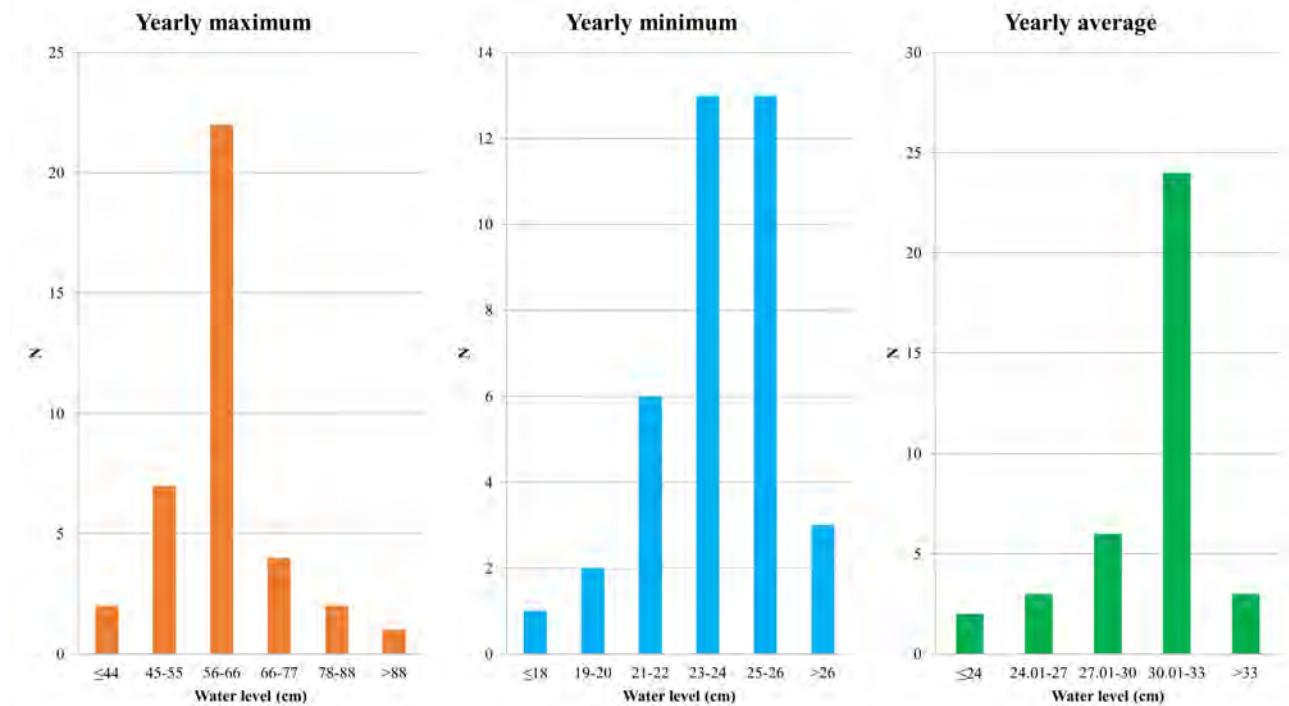


Figure 13: Histograms for yearly maximum, minimum and average water levels at station Koretići

### 3. Results and discussion

#### 3.1. Trends of meteorological variables

Meteorological data has shown that in the Zagreb aquifer area year 2014 was the year with the highest value of precipitation (**Table 1**), but also with the highest average air temperature and values of evapotranspiration and water available for infiltration. Years 2011 had the smallest values of precipitation, which is also associated with smallest values of evapotranspiration and water available for infiltration. In this year, maximum water available for infiltration was about 100 mm or less. In 2014 maximum water available for infiltration was about 800 mm which means that potential for water infiltration can vary a lot in different meteorological conditions. Evaluation of evapotranspiration and corrected evapotranspiration has showed that differences range from -9.9% to 22.9% with average of -3.3% (**Table 1**). The usage of exact variable in this case should be investigated in detail in the future research.

Monthly trend analysis has shown very interesting results. Precipitation trends resulted with no statistically significant trend, while statistically significant ascending trends for average air temperatures have been identified for February, April, June, July, August and November (**Figures 14 to 19; Table 2**).

**Table 1:** Yearly values of evapotranspiration and maximum water available for infiltration (modified according to Bačeković, 2020)

Year	Precipitation	Et	Et-corrected	Difference between Et and Et corrected (%)	Maximum water available for infiltration using Et	Maximum water available for infiltration using Et-corrected
1981	998.2	530.0	535.1	1.0	468.2	463.1
1982	877.8	518.2	550.8	6.3	359.6	327.0
1983	737.5	490.2	486.2	-0.8	247.3	251.3
1984	981.3	514.0	512.9	-0.2	467.3	468.4
1985	894.7	495.1	504.9	2.0	399.6	389.8
1986	955.4	511.8	537.7	5.1	443.6	417.7
1987	917.7	512.5	507.0	-1.1	405.2	410.7
1988	847.5	514.3	528.9	2.8	333.2	318.6
1989	989.1	542.3	666.6	22.9	446.8	322.5
1990	745.2	501.4	491.3	-2.0	243.8	253.9
1992	834.7	531.6	497.1	-6.5	303.1	337.6
1993	1039.3	546.0	559.3	2.4	493.3	480.0
1994	938	567.6	607.8	7.1	370.4	330.2
1995	1072	560.9	589.2	5.0	511.1	482.8
1996	1062.5	529.7	555.9	4.9	532.8	506.6
1997	877.9	522.9	538.0	2.9	355.0	339.9
1998	976.8	543.5	609.0	12.0	433.3	367.8
1999	1083.7	566.8	572.2	1.0	516.9	511.5
2000	785	536.7	539.0	0.4	248.3	246.0
2001	1021.2	567.4	583.2	2.8	453.8	438.0
2002	1049.3	585.6	630.4	7.7	463.7	418.9
2003	681.6	489.6	497.8	1.7	192.0	183.8
2004	971.8	543.8	562.6	3.5	428.0	409.2
2005	1102	545.7	610.4	11.9	556.3	491.6
2006	871.4	538.7	581.9	8.0	332.7	289.5
2007	992.3	582.8	602.8	3.4	409.5	389.5
2008	805.1	535.0	570.9	6.7	270.1	234.2
2009	872	551.6	538.0	-2.5	320.4	334.0
2010	1147.5	564.7	581.1	2.9	582.8	566.4

2011	560.3	444.4	471.9	6.2	115.9	88.4
2012	853.1	549.2	558.3	1.6	303.9	294.8
2013	1149.9	588.7	530.6	-9.9	561.2	619.3
2014	1459.5	653.1	690.5	5.7	806.4	769.0
2015	935.1	575.4	605.2	5.2	359.7	329.9
2016	973.9	568.6	586.5	3.1	405.3	387.4
2017	922	568.1	575.8	1.4	353.9	346.2
2018	968.3	587.0	584.5	-0.4	381.3	383.8
2019	1054.3	605.2	610.0	0.8	449.1	444.3

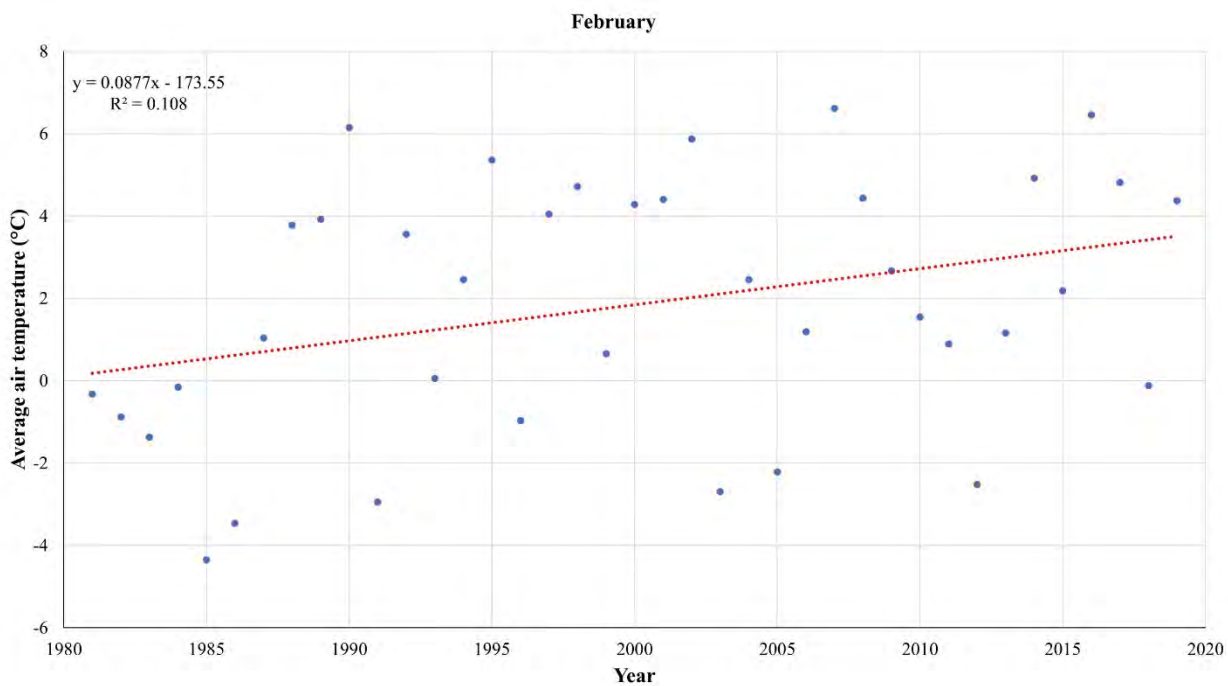


Figure 14: Trend of average monthly air temperature in February (modified according to Bačković, 2020)



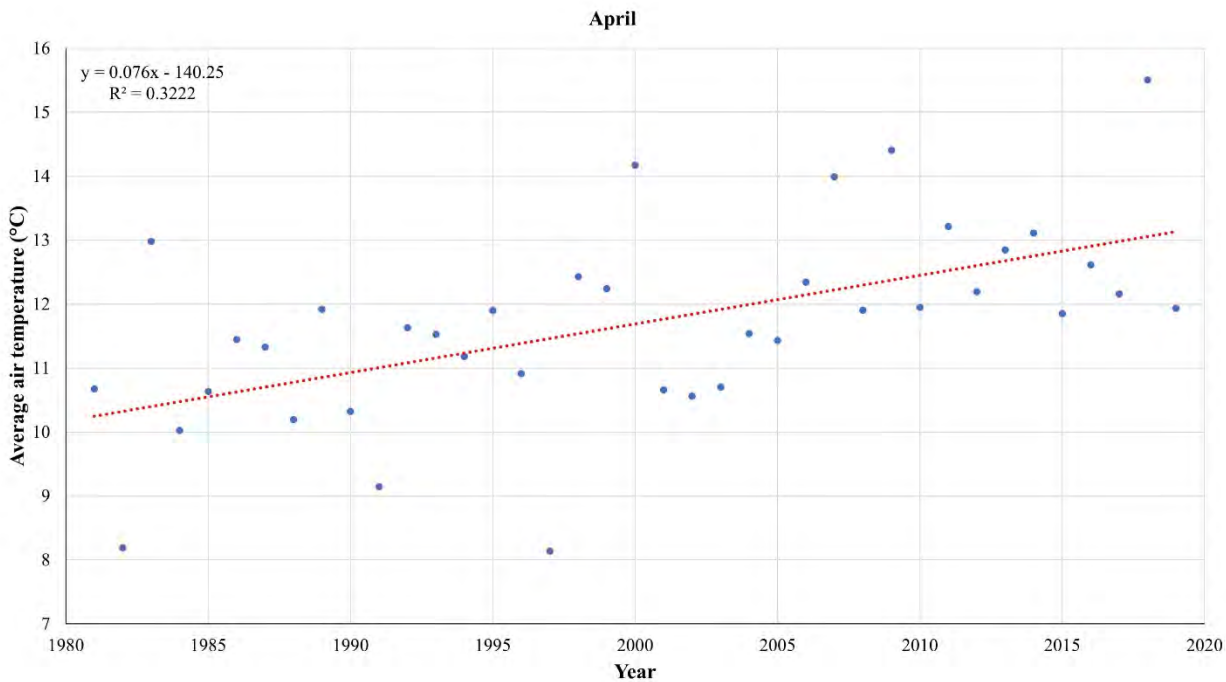


Figure 15: Trend of average monthly air temperature in April (modified according to Bačeko*vić*, 2020)

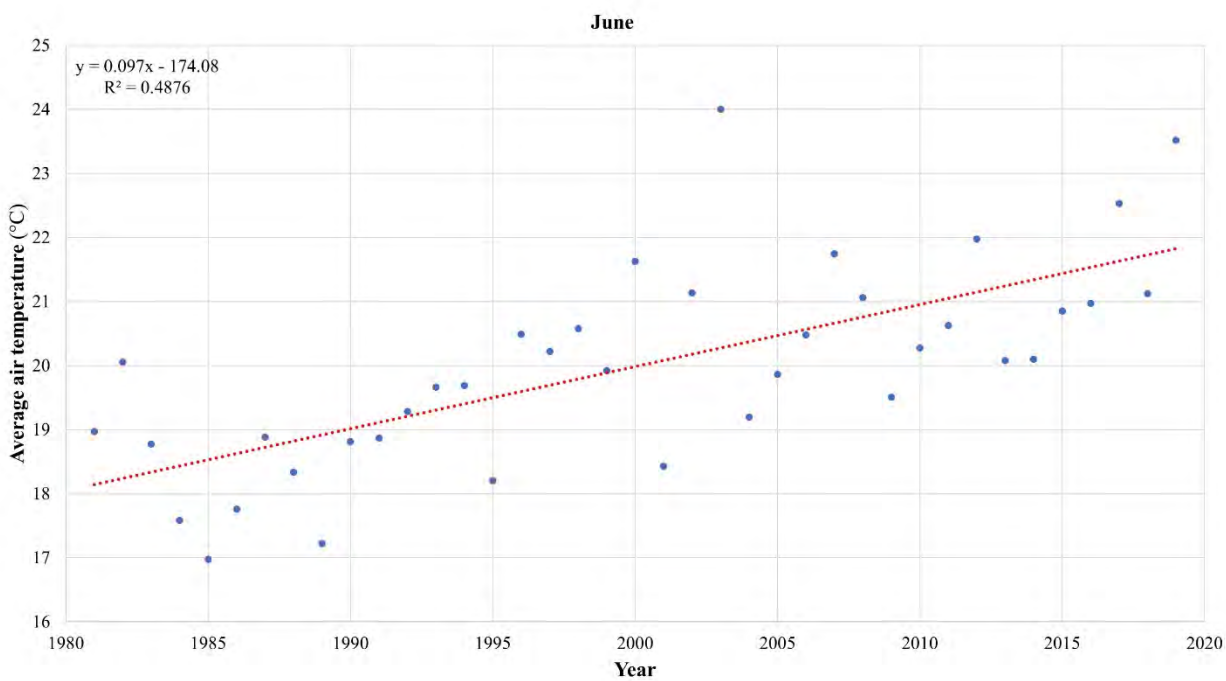


Figure 16: Trend of average monthly air temperature in June (modified according to Bačeko*vić*, 2020)

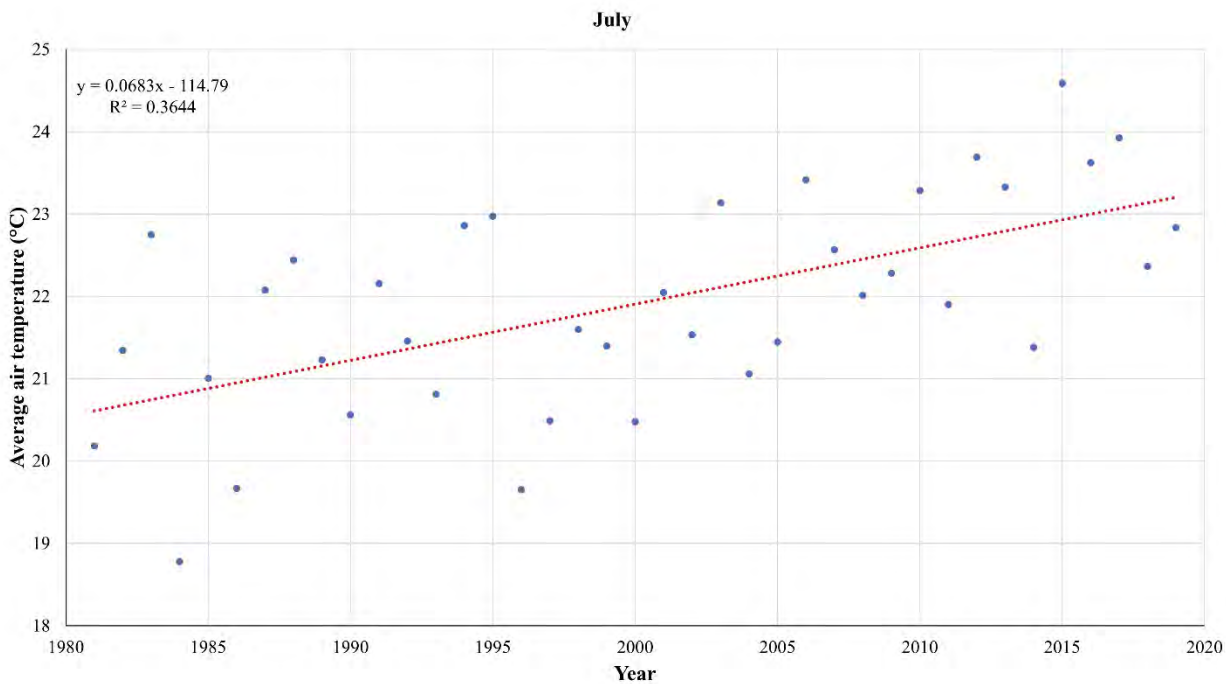


Figure 17: Trend of average monthly air temperature in July (modified according to Bačković, 2020)

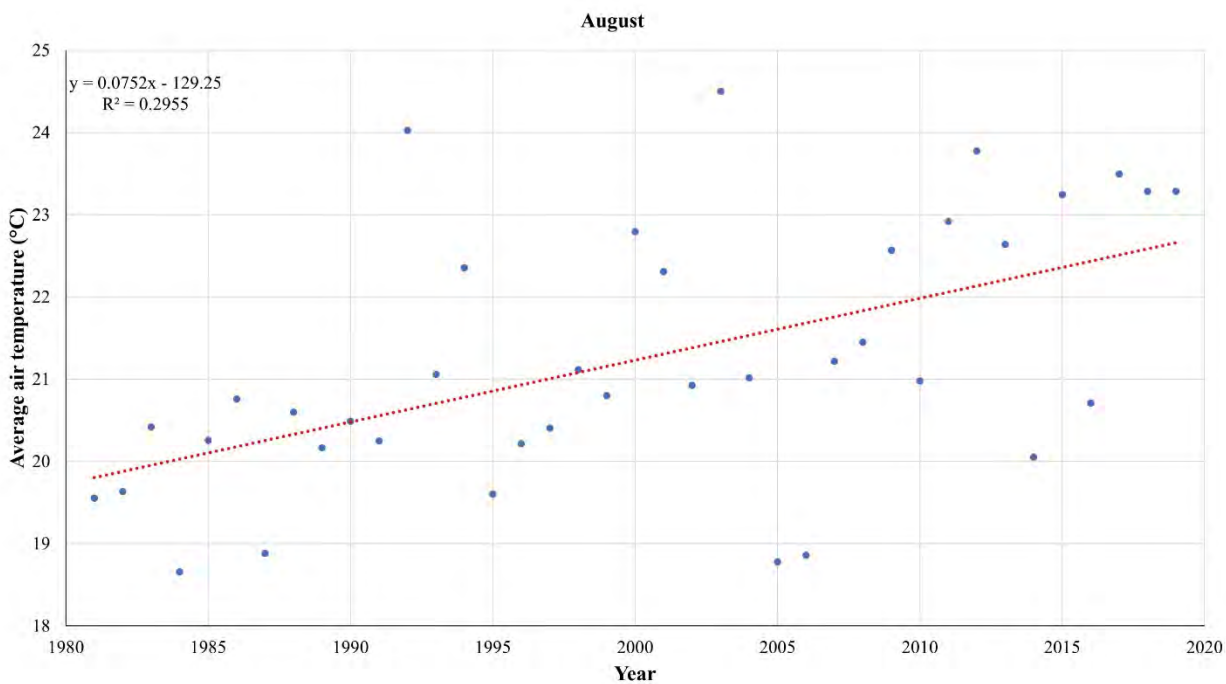


Figure 18: Trend of average monthly air temperature in August (modified according to Bačković, 2020)

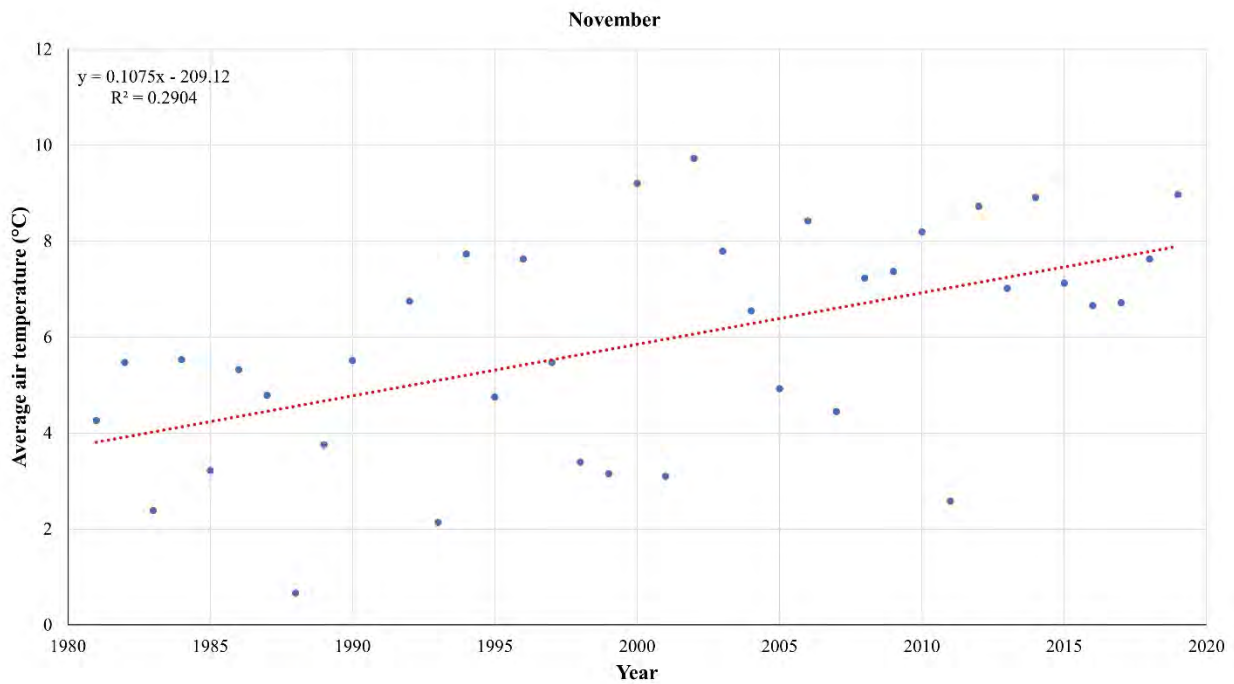


Figure 19: Trend of average monthly air temperature in November (modified according to Bačeković, 2020)

Table 2: Results of statistical analysis for monthly trends of precipitation and average air temperature (modified according to Bačeković, 2020)

Month	N	Precipitation			Average air temperature		
		p-value	statistically significant	trend	p-value	statistically significant	trend
January	39	5.17E-01	-	-	5.28E-02	-	-
February	39	1.22E-01	-	-	4.11E-02	+	ascending
March	39	2.54E-01	-	-	7.10E-02	-	-
April	39	5.14E-01	-	-	1.64E-04	+	ascending
May	39	5.45E-01	-	-	1.48E-01	-	-
June	39	9.95E-01	-	-	7.71E-07	+	ascending
July	39	2.86E-01	-	-	4.74E-05	+	ascending
August	39	3.74E-01	-	-	3.49E-04	+	ascending
September	38	9.85E-02	-	-	4.34E-01	-	-
October	38	8.84E-01	-	-	1.14E-01	-	-
November	38	2.94E-01	-	-	4.82E-04	+	ascending
December	38	3.36E-01	-	-	2.88E-01	-	-

When evaluating trends on a yearly basis, additionally, trends for evapotranspiration, corrected evapotranspiration and maximum water available for infiltration have been calculated. Similar to the results of a monthly analysis, precipitation trend is not statistically significant. Similar results for precipitation trends in the wider research area have been observed in the previous studies (Bonacci and Roje-Bonacci, 2019). On the other hand, trend of average air temperature is ascending, and is statistically significant. This also resulted in the statistically significant trends of evapotranspiration and corrected evapotranspiration, while for the maximum water available for infiltration, statistically significant trends have not been observed (Figure 20 to 22, Table 3).

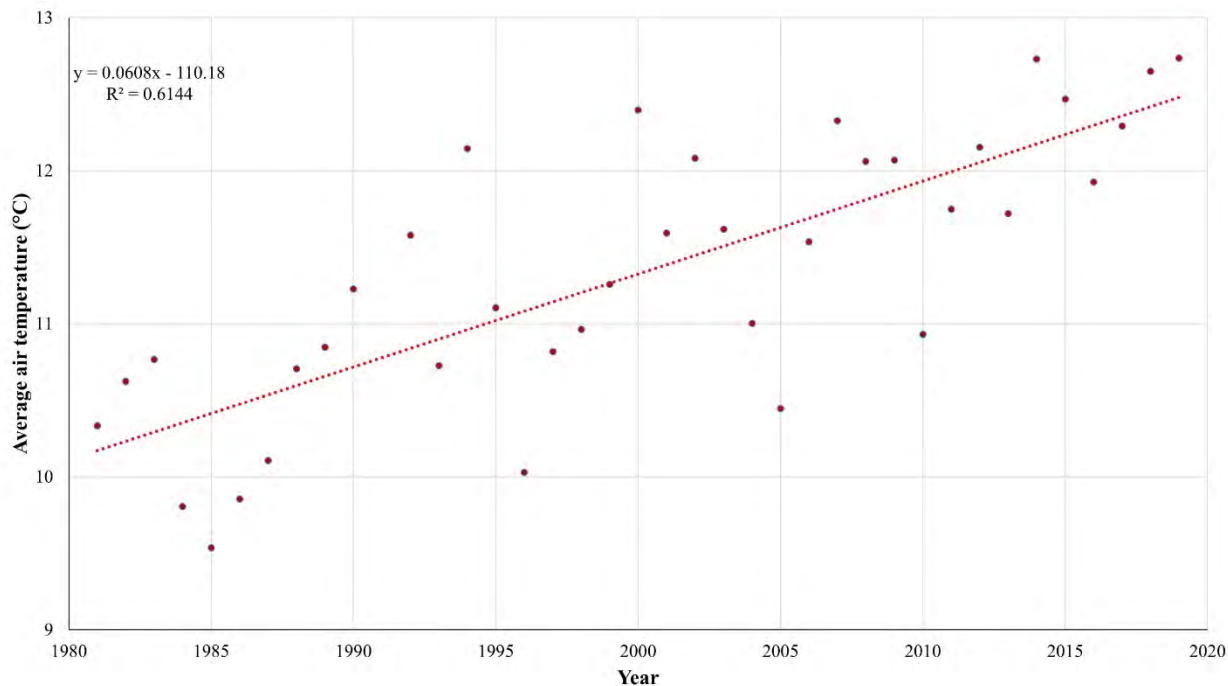


Figure 20: Trend of yearly average air temperature (modified according to Bačeković, 2020)

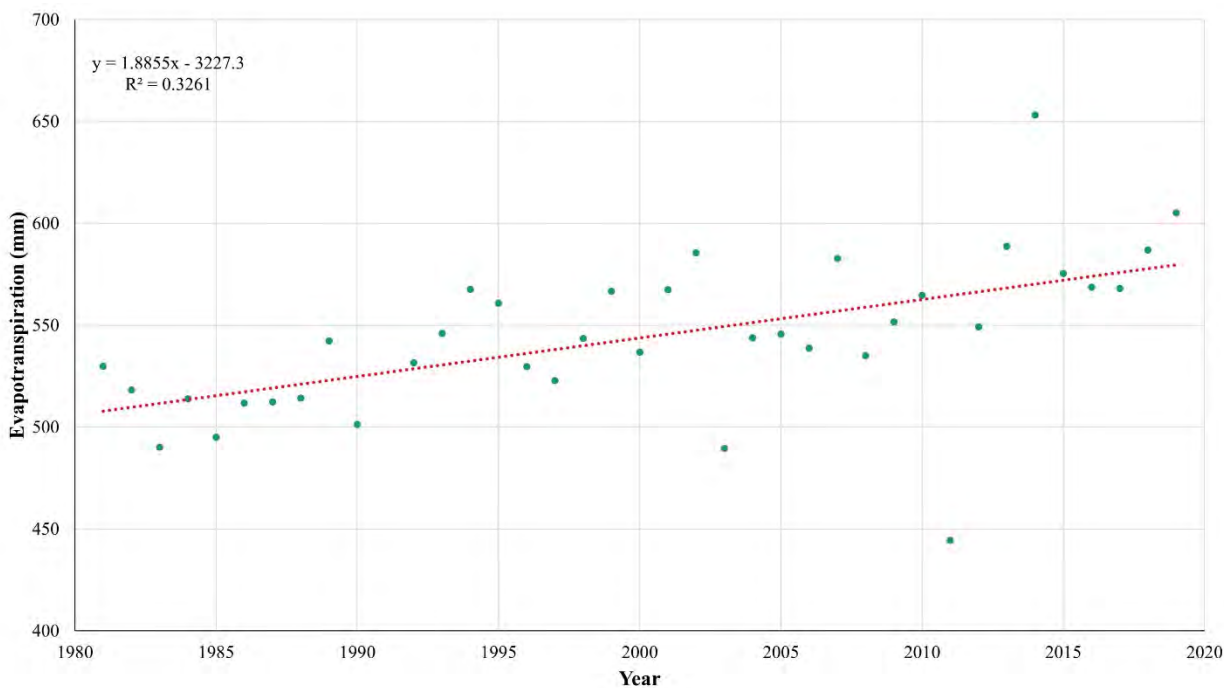


Figure 21: Trend of yearly evapotranspiration (modified according to Bačeković, 2020)



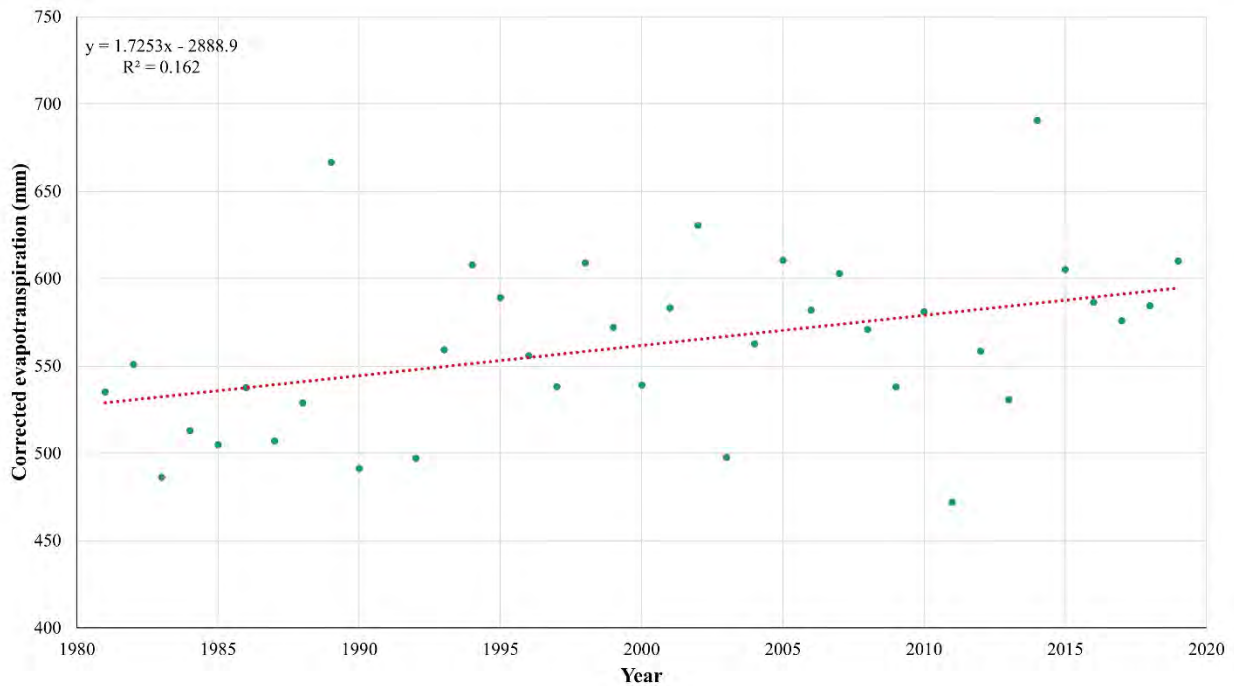


Figure 22: Trend of yearly corrected evapotranspiration (modified according to Bačković, 2020)

Table 3: Results of statistical analysis for yearly trends of precipitation, average air temperature, evapotranspiration, corrected evapotranspiration and maximum water available for infiltration (modified according to Bačković, 2020)

Variable	p-value	N	statistically significant	trend
Precipitation	2.14E-01	38	-	-
Average air temperature	5.88E-09	38	+	Ascending
Evapotranspiration	1.81E-04	38	+	Ascending
Corrected evapotranspiration	1.22E-02	38	+	Ascending
Maximum water available for infiltration	6.33E-01	38	-	-
Maximum water available for infiltration (calculated with corrected evapotranspiration)	5.68E-01	38	-	-

It can be seen that, when examining trends on a monthly and yearly basis, results are very similar. Trend of precipitation does not exist, neither in monthly or yearly calculation, while ascending trend of average air temperature in some months has generated the existence of yearly ascending trend in a whole examined period. Also, these results showed that evapotranspiration, and consequently maximum water available for infiltration, depends more on temperature than on precipitation, in a case when Turc’s formula is used for calculation. Although it’s not that obvious, if temperature continuous to grow, much less water for infiltration should be expected, especially in the summer months, where hydrological minimums occur in most cases. This suggests that in the future, part of the decrease in the seasonal groundwater reserves of the Zagreb aquifer, if observed, could be related with the climate change and warmer climate. However, this issue should be explored in detail in the future research.

### 3.2. Trends of river water level

Within the hydrological part of the trend analysis, trends have been calculated for the minimum, maximum and average values of Bregana River water level at hydrological station Koretići. Also, trends have been examined on a

monthly and yearly basis. Summary statistics for monthly and yearly trend analysis is shown in **Table 4**. Due to a lot of statistically significant trends, only trends calculated on a yearly basis have been graphically presented (**Figure 23 to 25**). It can be seen that only statistically ascending trend has been observed for the February when evaluating maximum water levels of the Bregana River. Although ascending trend for maximum values has been observed, it is not statistically significant, which is partly consistent with the previous research (**Ivezić et al., 2019**), where more pronounced ascending trend has been observed after year 2000. All other statistically significant trends are descending, especially when observing minimum water levels, both on monthly and yearly basis. Results suggest that in the future low waters caused by dry periods will be more pronounced. Another question that arises is how will this affect flooding of the areas downstream. Although, in general, ascending trends have not been observed, results suggest that bigger differences between minimum and maximum, i.e. extremes, of the Bregana River water levels can be expected (**Figure 26**). When considering all selected time period (1980 to 2017), results show ascending, but not statistically significant trend, what coincides with the yearly statistically significant descending trend for minimum and ascending, but not statistically significant trend for maximum water levels. However, it is evident that these values are slowly growing. For example, in last seven years average difference in cm was 43.43 cm, while from the 1980 till 2010 it was 35.42 cm. Although occasionally Bregana River floods areas downstream, results suggest that in the future dry periods will appear more often. On the other hand, results show that more frequent flooding can be expected in February.

If all results are compared together, it is evident that increase in air temperature and evapotranspiration will generate more dry periods what is consistent with the results of trend analysis of the Bregana River water level, which showed that in general descending trend should be expected. All these points suggest that less water from the precipitation, as well as reduced inflow to the Sava River from the Bregana River, will result with the smaller recharge to the Zagreb aquifer in the future, i.e. the smaller seasonal groundwater reserves, which could be because of the climate change.

**Table 4:** Summary of trend analysis for Bregana River water levels (modified according to **Rukavina, 2020**)

Month	N	Minimum			Maximum			Average		
		p-value	statistically significant	trend	p-value	statistically significant	trend	p-value	statistically significant	trend
January	37	4.24E-04	+	descending	1.33E-01	-	-	2.68E-03	+	descending
February	37	8.30E-03	+	descending	1.30E-02	+	ascending	8.40E-01	-	-
March	37	1.52E-02	+	descending	1.58E-01	-	-	8.18E-02	-	-
April	37	1.09E-03	+	descending	2.12E-01	-	-	2.99E-02	+	descending
May	37	1.75E-03	+	descending	8.68E-01	-	-	1.72E-02	+	descending
June	37	6.00E-04	+	descending	1.84E-02	+	descending	1.55E-04	+	descending
July	37	1.60E-03	+	descending	1.05E-01	-	-	6.93E-04	+	descending
August	37	4.20E-02	+	descending	4.10E-01	-	-	6.25E-02	-	-
September	38	1.69E-04	+	descending	6.22E-01	-	-	1.90E-02	+	descending
October	38	3.44E-04	+	descending	8.71E-01	-	-	8.14E-03	+	descending
November	38	1.79E-02	+	descending	1.94E-01	-	-	3.53E-01	-	-
December	38	3.40E-03	+	descending	7.56E-02	-	-	7.90E-03	+	descending
Yearly basis	38	1.04E-02	+	descending	4.94E-01	-	-	2.46E-04	+	descending

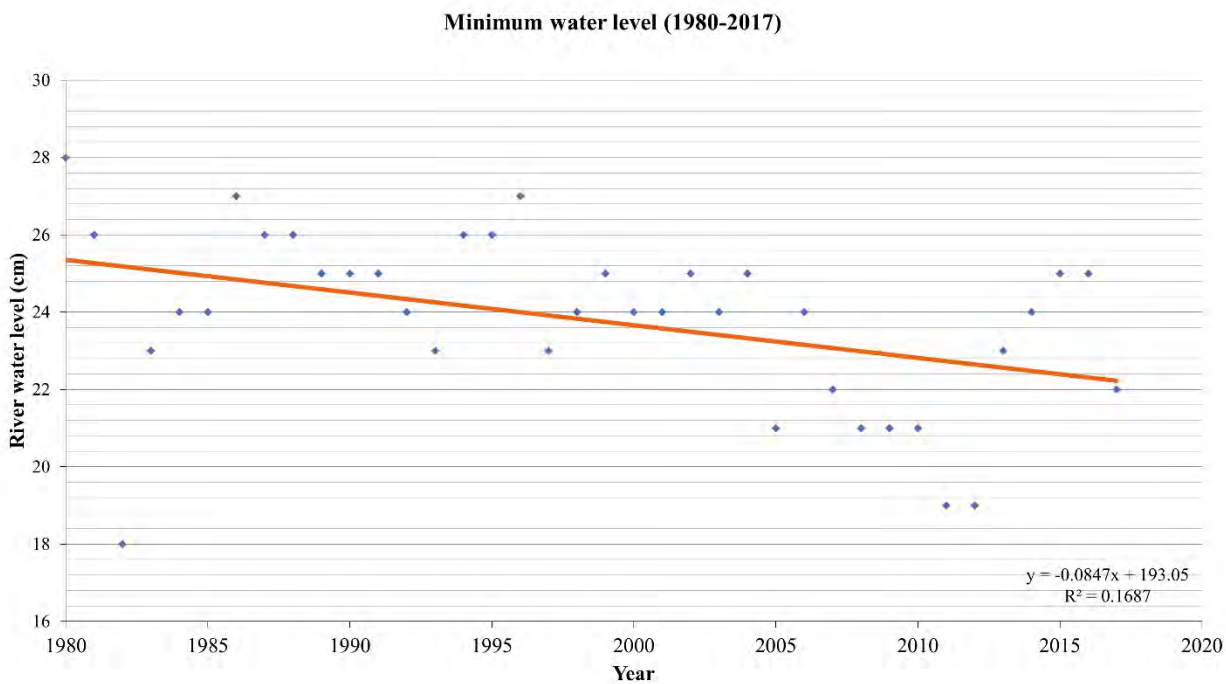


Figure 23: Trend of minimum water level of the Bregana River (modified according to Rukavina, 2020)

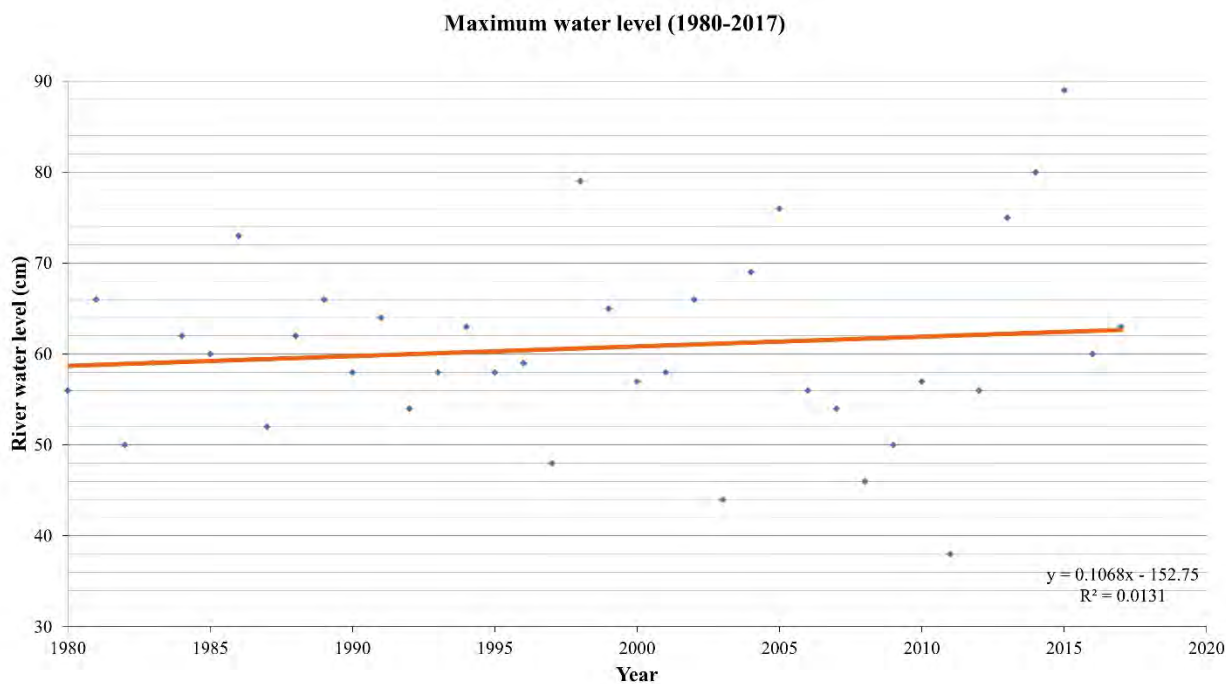


Figure 24: Trend of maximum water level of the Bregana River (modified according to Rukavina, 2020)

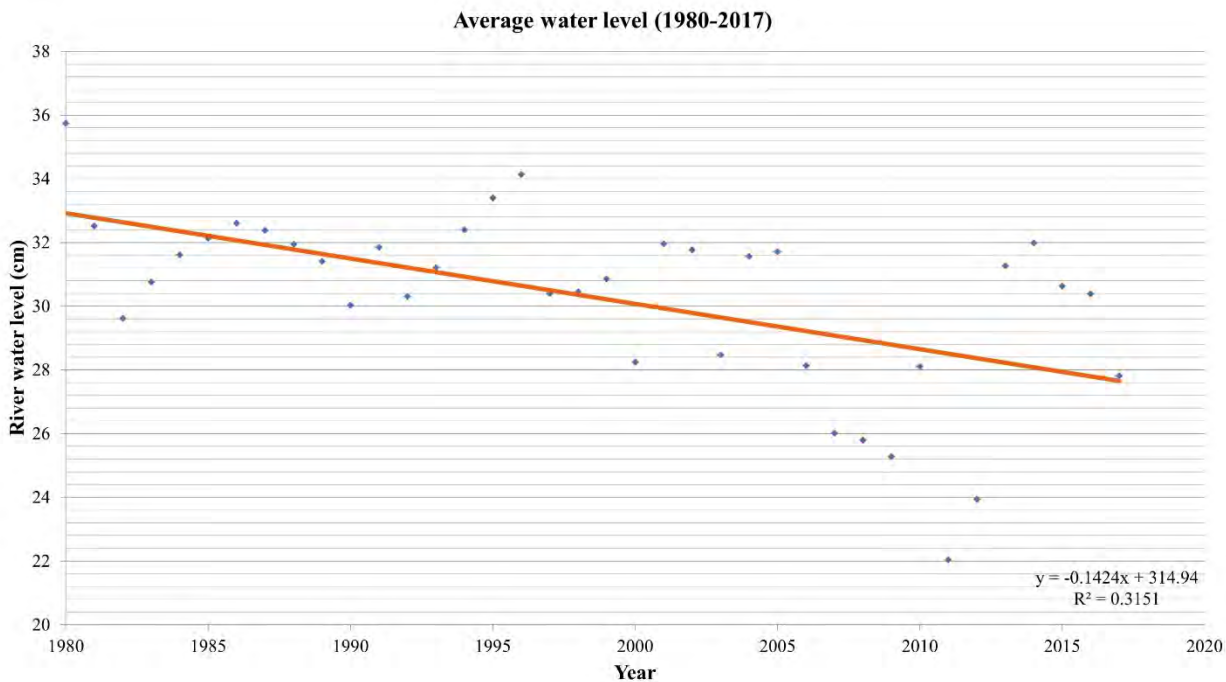


Figure 25: Trend of average water level of the Bregana River (modified according to Rukavina, 2020)

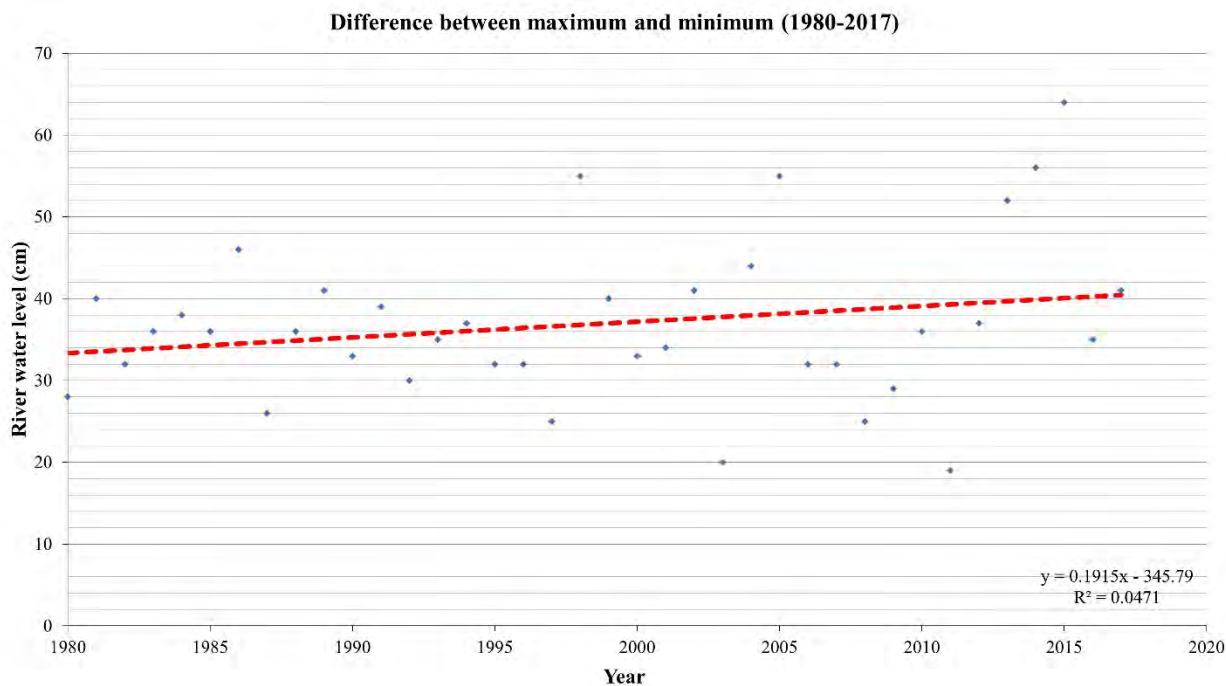


Figure 26: Trend of difference between minimum and maximum water levels of the Bregana River (modified according to Rukavina, 2020)



## 5. Conclusions

In this paper linear regression was used to estimate monthly and yearly trends of meteorological and hydrological variables. Statistical significance was tested with t-test ( $\alpha=0.05$ ). Monthly trend analysis showed statistically significant ascending trends for air temperature and evapotranspiration (especially in the summer months), and descending trends for Bregana River water levels when observing minimum and average data. Statistically significant ascending trends of Bregana River maximum water levels are only observed for the February, which could indicate that more flooding can be expected in that month. Although yearly trends showed similar pattern in most cases as monthly trends, it was also shown that sometimes evaluation of trends on a yearly basis could hide potentially very important information. This suggests that trends of hydrometeorological variables should be evaluated in the different time scales. Most of the results suggest that more dry periods should be expected, as well as bigger difference between low and high water, i.e. extremes. It is evident that smaller infiltration from precipitation and reduced inflow from Bregana River to Sava River will probably result with the smaller recharge to the Zagreb aquifer. All this suggests that climate change is potentially occurring in the research area. However, to confirm this, it is necessary to conduct more detail research with more observation points and longer time period. Also, future research must include hydrological and hydrogeological analysis and variables, for example evaluation of river water level duration curves and trend of groundwater levels.

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## SAŽETAK

### Trendovi hidrometeoroloških varijabli na širem području zagrebačkog vodonosnika

Cilj ovog rada bio je procijeniti trendove hidrometeoroloških varijabli u različitim vremenskim periodima kako bi se vidjelo hoće li rezultati generirati različite zaključke i postoje li naznake utjecaja klimatskih promjena na šire područje istraživanja. Trendovi su procijenjeni na mjesečnoj i godišnjoj razini za oborine, temperaturu zraka, evapotranspiraciju i maksimalnu vodu dostupnu za infiltraciju na meteorološkoj postaji Pleso, i za vodostaje rijeke Bregane na hidrološkoj postaji Koretići. Obje lokacije su važne jer mogu ukazati na obrasce koji bi mogli biti karakteristični za šire područje Zagreba, a povezani su sa napajanjem zagrebačkoga vodonosnika. Linearna regresija, zajedno s *t-testom*, korištena je za procjenu trenda. Mjesečna analiza trendova pokazala je rastuće trendove temperature zraka i evapotranspiracije, posebno u ljetnim mjesecima. Silazni trendovi vodostaja rijeke Bregane primijećeni su kada su uzete u obzir minimalne i prosječne vrijednosti, dok je statistički značajan uzlazni trend maksimalnih vodostaja uočen samo za veljaču. Općenito, godišnji trendovi pokazali su sličan obrazac. Također se pokazalo da evaluacija trendova samo na godišnjoj razini može ponekad sakriti važne informacije. Svi rezultati sugeriraju da bi trebalo očekivati više sušnih razdoblja, dok bi poplave mogle biti češće u veljači. Iako nije statistički značajna, primijećena je veća razlika između visokih i niskih voda, tj. ekstrema. Trendovi su pokazali kako se može očekivati manja infiltracija vode od oborina i smanjeni dotok rijeke Bregane u rijeku Savu, koji će vrlo vjerojatno rezultirati slabijim napajanjem zagrebačkoga vodonosnika, što potencijalno može biti posljedica klimatskih promjena.

**Ključne riječi:** trend; hidrometeorološka varijabla; zagrebački vodonosnik; linearna regresija

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### Author's contribution:

**Sara Bačeković (1)** (student) made the statistical analysis for meteorological variables, while **Dominik Rukavina (2)** (student) made it for hydrological variables. **Zoran Kovač (3)** (assistant professor) defined the concept and wrote first draft of the manuscript. All authors participated in the creation of figures and tables, and data interpretation.