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EFFECTS OF SILICON ON THE BIOCHEMICAL CHARACTERISTICS OF WHEAT UNDER DROUGHT STRESS CONDITIONS

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Abstract – In order to determine the effect of drought stress on the biochemical properties of wheat, it was investigated in a factorial experiment in the form of a randomized complete block design with three replications in the research field of the Soil and Water Research Institute in the crop year 2018-2019. and 2019-2020.The treatments included drought stress at three levels without stress, mild stress as the first factor. The second factor of Potassium silicate was investigated at four control levels of 0 and 20 kg/ha as soil application and foliar spraying application with concentrations of 2.5 and 5 kg/ha in Sivand wheat cultivar. The results of analysis of variance showed that the effect of drought stress on Malondialdehyde, Superoxide dismutase, Ascorbate peroxidase, Catalase, Glutathione peroxidase and plant growth was significant ($p<0.01$) and Proline traits and seed yield were significant 5% level ($p< 0.05$). The effect of Silicon on superoxide dismutase traits ($p<0.01$) and malondialdehyde, ascorbate peroxidase, catalase, glutathione peroxidase, breeding and seed yield was significant ($p<0.05$). The results of the treatment interaction showed that catalase and glutathione peroxidase traits were significant ($p<0.01$). As a result, the use of silicon by improving biochemical characteristics can help wheat to overcome drought stress.

Keywords: catalase; grain yield; proline; protein; wheat.

Introduction

Wheat constitutes the major food of most people in the world and accounts for the highest area under cultivation of agricultural lands worldwide. Owing to its unique properties, wheat is the most important crop on the globe (Tester & Langridge, 2010). In Iran, wheat also accounts for the highest area under cultivation, and this issue doubles the importance and the need for planning and optimal management of resources and wheat-producing factors (Emam, 2007). Crops are exposed to multiple environmental stresses, all of which affect their growth, thereby influencing crop production levels. Drought stress is among the most destructive stresses that reduce the productivity of crops more than other stresses (Majidi & Amiri, 2020). Drought is the most important factor limiting the growth and yield of crops and affects 40-60% of agricultural lands worldwide (Sinclair, 2011). High fluctuations are observed in the yields

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of successive years due to shortage and uneven distribution of precipitation from one year to another. Additionally, an increase in the evapotranspiration rate causes the incidence of drought stress during the growth period of plants (Gonzalez et al., 2010). To prevent the destructive effects of stress, plants use a complex defense system including enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase, ascorbate peroxidase (APX), and glutathione reductase (GR), as well as non-enzymatic antioxidants including ascorbic acid, glutathione, carotenoids, and tocopherol (Verma et al., 2015). With the incidence of environmental stresses, increasing both the activity of antioxidant enzymes and the content of osmolytes plays a vital role in tolerance to biotic and abiotic stresses in plants (Epstin, 1999). Gong et al. (2005) concluded that silicon (Si) application would increase the activity of antioxidant enzymes (SOD, CAT, and GR). Their results indicated that drought stress led to an increase in H_2O_2 levels whereas Si reduced H_2O_2 levels, acid phospholipase activity, and damages caused by oxidant stress.

The essential factor related to crop production is good plant nutrition, which plays a remarkable role in increasing yield. Accordingly, the role of some nutrients, such as Si, has been of interest to some plant nutrition researchers (Gong & Chen, 2012). Si is a nonessential but useful nutrient affecting the growth and health of plants. Most plants can absorb Si and the absorption rate is in the range of 0.1-10% of plant biomass depending on the plant type (Cherif & Belanger, 1992).

The effect of Si on plant yield may arise from its precipitation in the leaf width, increasing leaf strength (Adatia & Beasford, 1986), elevating chlorophyll concentration in the leaf unit area (Maghsoudi et al., 2016), and increasing photosystem II efficiency (Popovic et al., 2003). More chlorophyll concentration can improve photosynthesis in plants (Maghsoudi et al., 2016). An increase in tolerance to environmental stresses, including drought, by Si application has been reported in several studies (Maghsoudi & Emam, 2016).

Gong et al. (2005) investigated the effect of Si on the defense of wheat against oxidative stress under drought stress in different stages of growth and development. They reported that the use of Si increased plant water potential under drought stress at the filling stage, but this was not true for the heading stage. The researchers observed SOD inhibition and an increase in peroxidase (POX) activity at the pod filling stage by drought stress. At the grain filling stage, Si application resulted in an increase in SOD activity and a reduction in POX activity under drought stress. CAT activity was slightly elevated under drought stress, and Si application did not change H_2O_2 levels and soluble protein content at the heading stage under drought stress, but it led to a decrease in H_2O_2 concentrations and a rise in soluble protein content.

Ideal soil fertility is a major factor in increasing wheat production. Good plant nutrition is also a solution to reduce the detrimental effects of stresses and plays a marked role in reducing its yield. In this regard, the role of some nutrients, such as Si, has attracted the attention of some plant nutrition researchers (Gong & Chen, 2012). Therefore, this research was conducted to evaluate the effects of Si on agronomic and physiologic traits of the Sivand wheat cultivar in drought stress conditions.

Table 1. The results of mixed ANOVA for the effects of Si on biochemical traits of wheat in drought stress conditions.

SOV	df	Mean of squares				
		MDA	**SOD**	**APX**	**CAT**	**GPX**
Year	1	0.000272ns	3.104ns	0.00012ns	0.0000001ns	1610.00ns
Year repeat	4	0.002544	1.836	0.000311	0.0001488	3274.0*
Drought stress	2	0.912712**	588.844**	0.676901**	0.0083294**	4403403.00**
Year × stress	2	0.005676*	2.066ns	0.002625ns	0.0001059**	1956.00**
Si	3	0.0605*	95.831**	0.02609*	0.0005404*	385861.00*
Year × Si	3	0.00298*	0.328ns	0.000602ns	0.0000435**	412.00ns
Si × stress	6	0.000612ns	4.602*	0.003785*	0.0000361**	69046.00**
Year × stress × Si	6	0.000517ns	0.808ns	0.000579ns	0.0000022ns	159.0ns
Error	44	0.004294	3.91	0.000307	0.0000966	1104
cv (%)	***	9.71	11.7	4.09	22.7	5.35

*, **, and ns: significant difference at 5% and 1% probability levels and no significant difference, respectively.

Table 2. Comparison of average Si and drought stress on the biochemical traits of wheat.

Treatments		MDA (µmol.g)	SOD (µmol.g)	APX (µmol.g)	CAT (Unit.mg^{1}Protein)	GPX (µmol.g)
Drought stress	S1	0.481	11.35	0.255	0.027	138.778
	S2	0.871	18.513	0.437	0.039	764.96
	S3	0.672	20.858	0.591	0.064	958.177
	LSD	**0.107**	**3.25**	**0.028**	**0.016**	**54.68**
Si	SI1	0.753	13.643	0.38	0.038	454.946
	SI2	0.679	16.965	0.461	0.05	683.863
	SI3	0.649	18.219	0.415	0.04	554.379
	SI4	0.618	18.802	0.455	0.046	789.365
	LSD	**0.09**	**2.81**	**0.024**	**0.013**	**47.35**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

Materials and methods

This study was carried out as a randomized complete block design with three replications in the research field of the Soil and Water Research Institute in the 2018-2019 and 2019-2020 crop years. The geographic coordinates of the study area include 50° 57' E and 35° 45' N with an altitude of 1280 m from the sea level. In Alborz province, the climate falls into temperate to cold areas with an average precipitation of 250 mm. Experimental treatments were drought stress at three levels without stress, mild stress, and severe stress with 75, 50, and 25% usable moisture in the soil as the first factor. The second factor was potassium silicate at four levels of no Si application (control), 20 kg/ha as soil application at the bolting stage, and spraying at 2.5 and 5 kg/ha at bolting, heading, and seed dough stages, which were examined in Sivand wheat cultivar (drought-sensitive) for 2 years. Wheat plants were cultivated on December 10, and six planting lines (4 m and 20 cm in length and width, respectively) were determined in each plot.

Measurement of traits

Proline content (mg/g leaf fresh weight) accumulated in the leaves at the flowering stage was measured by spectrophotometry at 520 nm wavelength (Bates et al., 1973). CAT activity was determined according to Boominathan & Doran (2002) as described below. First, 900 µl of the reaction solution (containing a 10 mM H_2O_2 solution in PVP-free phosphate buffered saline and 100 µl of enzymatic extract) was poured into a cuvette. Then, H_2O_2 was added to the reaction solution, and the reduction caused by H_2O_2 decomposition by CAT activity was immediately measured with a spectrophotometer (Uvi Light XS 5 SECOMAM) at 240 nm wavelength for 1 min, followed by calculating CAT activity.

APX activity was assessed at the flowering stage according to Boominathan & Doran (2002). Initially, 900 µl of the reaction solution (625 µl of EDTA-containing phosphate buffer, 175 µl of ascorbic acid, 50 µl of H_2O_2, 50 µl of BSA, and 100 µl of enzymatic extract) was poured into a cuvette. Then, the reduction in ascorbic acid caused by the enzyme activity was measured with a spectrophotometer at 290 nm wavelength for 1 min, followed by measuring APX activity. SOD activity was measured through its ability in preventing the photoreduction of nitroblue tetrazolium (NBT) chloride at the flowering stage as described by Dhindsa et al., (1981). To this end, 3 ml of the reaction solution was prepared to contain 50 mM potassium phosphate, 13 mM methionine, 75 µM NBT chloride, ethylene in 0.1 mM tetraacetic acid, 360 µM riboflavin, and 30 µl of crude extract. After stirring the mixture, the spectrophotometer cells were exposed to a 15 W fluorescent light at a distance of 35 cm for 10 min. The reaction was stopped by turning off the light, and the mixture absorbance was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme that could prevent the photoreduction of NBT chloride by 50%. The specific activity of the enzyme was reported as the enzyme units in mg of protein.

The malondialdehyde (MDA) biomarker was determined using a previously reported method (Heath & Packer, 1986). Based on this method, 0.2 g of terminal young leaf fresh tissue was weighed and pulverized in a porcelain mortar containing 5 ml of 1% trichloroacetic acid (TCA). The resulting extract was centrifuged at 10,000 rpm for 5 min. Then, 4 ml of 40% TCA containing 0.5 thiobarbituric acid (TBA) was added to 1 ml of the supernatant. The resulting mixture was heated in a water bath at 95 C for 30 min, cooled immediately on ice, and re-centrifuged at 10,000 rpm for 10 min. The optical density of this solution was read at 532 nm using a spectrophotometer. The MDA-TBA red complex is the substance of interest for absorbance in this wavelength. The absorbance of other non-

Table 3. Comparison of the average effect of year × Si and drought stress on the biochemical traits of wheat.

Treatments			MDA (µmol.g)	SOD (µmol.g)	APX (µmol.g)	CAT (Unit.mg^{1}Protein)	GPX (µmol.g)
Year × stress	Y1	S1	0.478	11.523	0.263	0.026	135.085
		S2	0.861	19.03	0.424	0.038	750.729
		S3	0.692	20.791	0.592	0.066	961.913
	Y2	S1	0.484	11.177	0.248	0.029	142.472
		S2	0.882	17.997	0.45	0.04	779.191
		S3	0.653	20.925	0.59	0.061	954.441
	LSD		**0.07**	**2.3**	**0.02**	**0.011**	**38.66**
Year × Si	Y1	SI1	0.769	13.665	0.371	0.038	456.856
		SI2	0.664	17.31	0.466	0.047	678.504
		SI3	0.65	18.437	0.416	0.041	548.393
		SI4	0.624	19.047	0.453	0.047	779.883
	Y2	SI1	0.738	13.62	0.388	0.038	453.037
		SI2	0.694	16.62	0.456	0.052	689.222
		SI3	0.649	18.001	0.413	0.039	560.366
		SI4	0.611	18.557	0.458	0.045	798.847
	LSD		**0.06**	**1.99**	**0.017**	**0.009**	**33.48**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

specific pigments was determined at 600 nm and subtracted from this value. The concentration of this biomarker was calculated using a 1.56×10^5 extinction coefficient, and the measurement results were calculated based on µmolg^{-1} F. Seed protein content was obtained by multiplying seed nitrogen (%) by 6.25 (Jones et al., 1991).

To measure grain yield in individual plots, 0.5 m was eliminated from the initial and end of lines. Then, all spikes in three middle lines were harvested manually along 2 m. Grains were dried in an oven and then separated manually (Alavi Fazel, 2015).

Statistical analysis of data

The uniform variance of experimental errors was verified using Bartlett's test by Minitab software. Statistical calculations were done by MSTATC and Minitab software. Mean values were compared by the least significant method at the 5% probability level.

Results

According to the results in the mixed ANOVA table, MDA concentrations and the effects of Si, year × stress, and year × Si under drought stress were significant at 1% and 5% probability levels, respectively. However, the interaction of drought stress × Si was not statistically significant. As shown in the comparison of means (Table 2), the highest (0.871 µmol.g) and lowest (0.481 µmol.g) MDA concentrations were recorded under mild and no drought stress (control) conditions, respectively. The plants in treatments without Si application and 5 kg of Si spraying contained the highest (0.753 µmol.g) and lowest (0.618 µmol.g) MDA concentrations, respectively (Table 2), suggesting the destruction of the plant cell membrane under drought stress.

SOD activity

Based on the results of mixed ANOVA (Table 1), SOD activity was significantly affected by drought stress and Si treatments at the 1% level, and the interaction of drought stress × Si was statistically significant at the 5% level. According to the comparison of means (Table 2), the highest (20.85 µmol.g) and lowest (11.35 µmol.g) SOD activities belonged to severe stress and no drought stress (control) conditions, respectively. The highest (23.52 µmol.g) and lowest (8.14 µmol.g) SOD activities were obtained in severe stress treatments with 5 kg of Si spraying and no Si application without drought stress, respectively (Table 4).

APX activity

The results of mixed ANOVA (Table 1) revealed that APX activity was significantly influenced by drought stress at the 1% level. Si treatments and the interaction of drought stress × Si were statistically significant at the 5% level. As shown in the comparison of means (Table 2), the highest (0.591 µmol.g) and lowest (0.255 µmol.g) APX activities were measured in severe stress and no drought stress (control) conditions, respectively. Si soil application (20 kg) and no use of Si resulted in the highest (0.461 µmol.g) and lowest (0.38 µmol.g) APX activities. The interaction of treatments led to the highest (0.621 µmol.g) and lowest (0.175 µmol.g) APX activities using Si soil application (20 kg) in severe stress and no use of Si in stress-free conditions (Table 4).

CAT

According to the results of mixed ANOVA (Table 1), CAT concentrations were significantly affected by the effects of drought stress and the interaction of year × stress, year × Si, and drought stress × Si at 1% and 5% probability levels, respectively. The comparison of means (Table 2) showed that the highest (0.064 Unit.mg^{-1} Protein) and lowest (0.027 Unit.mg^{-1} Protein) CAT concentrations were measured under severe and no drought stress conditions, respectively. The plants in treatments with Si soil application (20 kg) and no Si spraying contained the highest (0.05 Unit.mg^{-1} Protein) and lowest (0.038 Unit.mg^{-1} Protein) CAT concentrations, respectively (Table 2). The interaction of drought stress × Si using 20 kg of Si soil application resulted in the utmost CAT concentration (0.069 Unit.mg^{-1} Protein) (Table 4). In this study, drought stress led to an increase in CAT activity. Drought stress increases ROS, thereby elevating antioxidant defense (Apel & Hirt, 2004).

Glutathione peroxidase (GPX)

Based on the results of mixed ANOVA (Table 1), GPX concentrations were significantly influenced by the effects of drought stress and the interaction of drought stress × Si at the 1% probability level, and the effect of Si was significant at the 5% level. The comparison of means (Table 2) indicated that the highest (958,177 µmol.g) and lowest (138.778 µmol.g) GPX concentrations belonged to severe and no drought stress conditions, respectively. The treatment with 20 kg/ha of Si spraying and Si-free treatment resulted in the highest (789.365 µmol.g) and lowest (454.946 µmol.g) GPX concentrations, respectively (Table 2). The interaction of treatments produced the highest (1100.13 µmol.g) and lowest (60.89 µmol.g) GPX activities in 20 kg/ha of Si spraying and Si-free treatments in severe and no drought stress conditions, respectively (Table 4).

Protein

As indicated by the mixed ANOVA table, protein content was significantly influenced by drought stress treatments and Si application at 1% and 5% levels (Table 5). A comparison of means revealed that protein content was affected by

Table 4. Comparison of the average interaction effect of Si and drought stress on the biochemical traits of wheat.

Treatments			MDA (µmol.g)	SOD (µmol.g)	APX (µmol.g)	CAT (Unit.mg^{1}Protein)	GPX (µmol.g)
Si × stress	S1	SI1	0.567	8.146	0.175	0.024	60.893
		SI2	0.492	12.39	0.284	0.032	130.913
		SI3	0.453	12.204	0.245	0.025	125.877
		SI4	0.413	12.66	0.318	0.028	237.43
	S2	SI1	0.957	15.45	0.395	0.032	482.218
		SI2	0.87	18.855	0.479	0.048	939.743
		SI3	0.843	19.525	0.421	0.034	607.345
		SI4	0.815	20.223	0.453	0.043	1030.533
	S3	SI1	0.737	17.332	0.569	0.058	821.727
		SI2	0.677	19.65	0.621	0.069	980.933
		SI3	0.652	22.928	0.578	0.06	929.917
		SI4	0.625	23.522	0.596	0.068	1100.132
	LSD		**0.05**	**1.62**	**0.014**	**0.008**	**27.34**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

severe and no stress treatments at the highest (14.60%) levels. Treatments with 5 kg and no Si application led to the highest (12.64%) and lowest (10.16%) effects, respectively, on protein content (Table 6).

Proline

The results of mixed ANOVA (Table 5) indicated that proline content was significantly affected by drought stress treatments at the 5% level. A comparison of means showed that proline content was affected by severe and no drought stress treatments at the highest (15.56 mg·g) and lowest (7.42 mg·g) levels (Table 6).

Grain yield

According to the mixed ANOVA table, grain yield was significantly affected by drought stress treatments and Si application at the 5% level (Table 5). A comparison of means showed that severe and no drought stress treatments exerted the lowest (4931.33 $kg.ha^{-1}$) and highest (6872.16 $kg.ha^{-1}$) effects on grain yield. Treatments with 5 kg and no Si application led to the highest (12.64%) and lowest (10.16%) effects, respectively, on grain yield (Table 5).

Discussion

In this study, considering the water shortage crisis and saving water resources and on the other hand, the important role of silicon in reducing the negative effects of drought stress, we investigated the effect of silicon on biochemical characteristics of wheat by applying drought stress. According to the results obtained from the analysis of variance and comparison tables, it can be stated, insufficient water supply at the vegetative growth stage influences plant establishment and development, stem growth, and reduces substance accumulation in these organs (Aslam et al., 2015). In drought stress conditions, SOD makes up the first line of defense against active oxygen radicals in the cell and catalyzes the reduction of the superoxide radical to H_2O_2 and molecular oxygen. In the next step, the resulting H_2O_2 is scavenged by APX and CAT enzymes (Amini, 2014). APX and SOD antioxidants can directly react with the superoxide radical and other reactive oxygen species (ROS), which can reduce the damage severity (Israr & Sahi, 2006).

Our results indicate an increase in APX activity in stress conditions as the transcription of some genes related to antioxidant enzymes (e.g., GPX or APX) increases in drought stress conditions to improve plant status, which plays an important role in reducing ROS and the resulting damage (Marivani et al., 2019). Similarly, Kavas et al. (2013) reported that an increase in APX activity in drought stress conditions could inhibit ROS, including H_2O_2, which accumulates during stress.

In this study, drought stress led to an increase in CAT activity. Drought stress increases ROS, thereby elevating antioxidant defense (Apel & Hirt, 2004).

High protein content in drought stress conditions relative to optimum situations can be associated with the reduced duration of growth and development in water-limited treatments, leading to a reduction in the carbohydrate-to-protein ratio and an increase in protein content. Thalooth et al. (2006) reported that deficit irrigation stress resulted in the impaired photosynthesis process, the activity of enzymes, and protein synthesis, affecting the transfer of metabolites to seeds.

Proline plays an essential role in osmoregulation, and the rise of proline content in plant tissues somehow indicates the activation of the osmoregulation mechanism, which provides the ground for more uptake of water and elements from the root environment (Munns, 2002). An increase in leaf proline content in drought stress conditions may result from protein degradation and decomposition of the carbon making up the leaf structure of the plant (Zadehbagheri et al., 2012).

Paknezhad et al. (2017) presented evidence that drought stress at reproductive stages led to reductions in most traits of yield and yield components compared to the control treatment, and the utmost yield reduction was observed in a drought stress treatment applied at the flowering stage. Based on the findings of Shahdi Komele & Kavousi (2004), Si significantly increased the rice plant yield by improving the morphological status and altering the chemical composition of this plant. To explain the mechanism of this result, they deduced that Si could reduce the toxicity of microelements and adjust the uptake of macroelements, thereby affecting the growth, yield, and weight of plant shoots.

Conclusion

Oxidative stress is a consequence of drought stress in plants. Antioxidant enzymatic activities often increase in plant cells exposed to environmental stresses, and thus plants can reduce the damage of created oxygen free radicals. Accordingly, drought stress application reduced grain yield in this research. The application of Si spraying (5 kg/ha) in stress-free conditions increased grain yield by 39% compared to the control.

Table 5. Two-year mixed ANOVA for the effects of Si on the yield and some biochemical traits of wheat under drought stress conditions.

SOV	df	Mean of squares		
		Protein	**Proline**	**Grain yield**
Year	1	0.629ns	24.21ns	1423547ns
Year repeat	4	0.377	1.289	35345
Drought stress	2	246.936**	425.905*	25933506*
Year × stress	2	2.311ns	4.379ns	470570ns
Si	3	23.141*	46.14ns	9309636*
Year × Si	3	1.624ns	1.335ns	533459ns
Si × stress	6	1.396ns	0.52ns	290284ns
Year × stress × Si	6	0.809ns	1.366ns	291215ns
Error	44	0.415	1.243	216071
cv(%)	***	5.51	10.26	8.17

*, **, and ns: significant difference at 5% and 1% probability levels and no significant difference, respectively.

Table 6. Comparison of average silicon and drought stress on grain yield and some biochemical traits of wheat.

Treatments		**Grain yield (kg.ha^{-1})**	**Protein (%)**	**Proline (mg.g)**
Drought stress	S1	6872.167	8.255	7.421
	S2	5256.333	12.222	9.619
	S3	4931.333	14.604	15.564
	LSD	**764.9**	**1.06**	**1.83**
Si	SI1	4758.222	10.163	8.901
	SI2	6044.889	12.453	10.714
	SI3	5525.111	11.514	11.049
	SI4	6418.222	12.644	12.808
	LSD	**662.4**	**0.91**	**1.58**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

Table 7. Correlations between yield traits and some biochemical traits of wheat due to silica and drought stress.

Traits	Grain yield	Protein	Proline	MDA	SOD	APX	CAT	GPX
Grain yield	1							
Protein	-0.528	1						
Proline	-0.395	0.900**	1					
MDA	-0.742**	0.455	0.110	1				
SOD	-0.495	0.951**	0.854**	0.478	1			
APX	-0.584*	0.966**	0.922**	0.433	0.903**	1		
CAT	-0.509	0.922**	0.937**	0.242	0.814**	0.947**	1	
GPX	-0.553	0.944**	0.840**	0.552	0.932**	0.928**	0.872**	1

*,**: represent significant differences at 5% and 1% levels, respectively.

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ENVIRONMENTAL FACTORS INFLUENCING *LOLIUM TEMULENTUM* L. (DARNEL RYEGRASS) SEED GERMINATION

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Abstract – Darnel ryegrass (*Lolium temulentum* L.) is an annual long-day plant belonging to the Poaceae family, that is common in grain fields worldwide. *L. temulentum* is a valuable model grass species for studying stress in forage and turf grasses. This study was conducted to assess the impact of critical environmental factors (temperature, light, pH, and salinity) on the seed biology of *L. temulentum*. The findings of this study indicated that the seeds of this weed germinate after three days at temperatures between 20 °C and 30 °C, 14 days at 10 to 15 °C, and 28 days at 5 ° C. Furthermore, at a temperature of 35 °C to 40 °C, the seeds did not germinate for 28 days. After 14 days, this species' most significant germination percentage was 91.6 % at 10 °C and 20 °C. Seeds did not germinate when incubated for 14 days under continuous darkness, whereas germinated at 32.5 % when subjected to light for 12 h daily. Also, the results showed no significant effect of examined pH (4-10) and salinity (0, 25, 50, 100, 150, 200, and 250) levels on seed germination of *L. temulentum*. The information gained from the analysis will provide a valuable biological plant germination resource that will be used to develop approaches as to how the plant can improve under abiotic stress factors.

Keywords: light, pH, salinity, temperature, weed.

Introduction

Darnel Ryegrass (*Lolium* spp.) is a bothersome weed in agricultural production globally, and it is moreover a valued cover crop, turf and cultivated forage species. High adaptive potential and diversity are identified to contribute to its achievement as a weed species and generate difficulties in precise species identification in fields (Maity et al., 2021). The genus of *Lolium* (Poaceae) comprises of several perennial and annual species. *Lolium* spp. are native to North Africa, Europe and temperate Asia, but have spread over the last 200 years to North and South America, New Zealand, Australia and southern parts of Africa. *Lolium* spp. were moved to new ares mainly as turf, pasture plants and cover crops, in contaminated livestock feed and commercial crop seed. Morphology of *Lolium* is very similar among members of the genus (Matzrafi et al., 2021).

One of the *Lolium* species *Lolium temulentum* L. is of global agricultural significance as both weeds and as pasture crops. Furthermore, it is a significant irritant in developing countries, and it is included among the 'Worst Weeds' in the world (Senda & Tominaga, 2004). The succulent weed *L. temulentum* originated in the Mediterranean and spread to temperate countries where wheat and grains are grown. Its spread into tropical regions in many countries is hindered by extended periods of elevated temperatures and little humidity (Holm et al., 1991). Due to a lack of studies, there is not much information on this plant's seed germination and emergence. Like other grass weeds of winter crops in temperate regions, it reproduces by seed. Low temperatures and high soil moisture promote its germination and growth; however,

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it can withstand extremely low temperatures (Holm et al., 1991). *L. temulentum* also failed to emerge at 10 cm seeding depth, according to Tanveer et al. (2010), and its emergence was reduced to 1 cm depth. Overgrown weeds in orchards are particularly troublesome in the first few years of a tree's existence. If the presence of herbs is not adequately handled, it can have severe repercussions since weeds can increase the insect activity and cause a risk of fire in the summer when conditions are dry (Sakit Alhaithloul, 2019).

Environmental stress factors have important effects on the growth of weeds. Temperature, one of the environmental stress factors, adversely affects plants in several ways, including plant germination, biomass, flower, and seed development. During heat stress, elements such as proteins, membranes, and mitochondria in plant cells can be damaged. With the effect of temperature stress, changes in photosynthesis, water and nutrient uptake, and changes in evapotranspiration are observed in plants. Similarly, lighting is a factor that can directly affect the photosynthetic activities and development of plants (Martin et al., 2021).

Lighting also plays a vital role in the development of weeds. The stress factor of light has a crucial role, especially in the germination phase. Although the effect of light on the germination rate varies according to the weed species, it also affects the *L. temulentum*, and the factor of light can disrupt the weed seed dormancy. Along with some studies, an attempt has been made to prevent the lighting factors that stimulate the germination of weeds by applying mulch (Botto et al., 1998; Kegode et al., 1998; Singh & Singh., 2009; Zimdahl, 2018).

Due to the lack of studies on biology and the effect of environmental conditions on seed germination, this work was conducted to study the impact of some environmental factors (temperature, light, pH, and salt stress) on the seed germination of the *L. temulentum*. With the change in the soil's pH level, there can be differences in the existing plants in the area and the species that will come to the area where the change is experienced. When there is a pH change, the competitive situation among plants can also change (Singh & Singh., 2009). So, it can be said that pH is effective in the formation of weed flora and the germination rate of weed species in a region (Alm et al., 1993; Forcella, 1993; Singh & Singh., 2009). The stress factor of salinity, which is one of the stress factors, also affects the development and survival of plants. The salinity factor affects plant growth and development as it causes a nutritional imbalance with excessive intake of ions such as sodium (Na^+) and chloride (Cl^-) (Isayenkov & Maathuis, 2019).

Our objective was to determine the effects of environmental factors (light, temperature, pH and salinity levels) on seed germination of *L. temulentum* populations. The results of the study would contribute to develop suitable and effective management strategies against the weed species.

Materials and Methods

Experimental site

This study was conducted to examine the effect of various environmental factors on *Lolium temulentum* L. seed germination. This weed's seeds were collected in the summer of 2020 from naturally ripened plants in date palm orchards in Southern Iraq; seeds were put in paper bags and stored in the lab at 20-25 °C until the experiment began. The study took place at Erciyes University Faculty of Agriculture in Plant Protection Department Herbology Laboratories in Kayseri –Türkiye from November 2020 to March 2021.

General seed germination tests

Seed preparation

L. temulentum seeds collected from Iraq agricultural lands were brought to Erciyes University laboratories in Türkiye. Those that are not healthy and damaged from the seeds were removed, they were not used in the experiment. In the experiment, seeds with a smooth appearance and no damage were preferred.

The seeds are stripped of any appendages or other items that must be removed. The seeds were cleansed once again, and any broken or rotting seeds and any pollutants overlooked in the previous phase were removed in this step. After that, the seeds were sterilized for 15 minutes in a safety cabinet with fluctuating UV light (Equipped by Berner International GmbH, Germany).

Germination of seeds

In order to examine the ecology of the seeds, *L. temulentum* seeds were subjected to ecological tests. Before testing the seeds, 30 *L. temulentum* seeds counted for each Petri dishes dish were kept separately in sodium hypochlorite solution (1%) for 1 minute and then washed 5-6 times with distilled water. Seeds were placed in Petri dishes (9 cm in diameter) containing double filter paper (Whatman No. 1), and then 5 ml of distilled water was added to the dishes. Petri dishes were covered with parafilm, since it is thought that there will be moisture loss from the Petri dishes with the temperature. Seed germination was examined at 1, 3, 7, 14, and 28 days with the criterion for germination being visible protrusion of the radicle (Göncü, 2013). Seeds were considered germinated when the radicle had emerged > 2 mm, and the radical protrusion was used to determine seed germination (Isik et. al., 2016).

Environmental conditions

Temperature

There is a certain temperature requirement during the germination period, which is the first stage of the development of plants. To determine the temperature required for the germination of *L. temulentum* seeds, different temperatures were applied to the seeds.

For 28 days, three replications of Petri dishes containing 30 sterilized seeds were placed in an incubator at varying temperatures (5, 10, 15, 20, 25, 30, 35, and 40 °C). The water level was frequently monitored to avoid drought, especially at elevated temperatures. The germination percentage was determined after 1, 3, 7, 14, and 28 days (Göncü, 2013). Germination percentages are calculated on the total of seeds put in Petri dishes at day 0. After the counts were completed, germination rate (G_{max}) and duration (T_{10}, T_{25}, T_{50} and T_{90}) values were calculated. According to this:

$$G_{max} = (G / T) \times 100$$

where G means number of total germinated seeds,
T means number of total seeds in experiment
T_{10}=Time to %10 of G-max or germinated seeds; T_{25}=Time to %25 of G-max or germinated seeds; T_{50}=Time to %50 of G-max or germinated seeds; T_{90}=Time to %90 of G-max or germinated seeds.

Light

The effect of light on seed germination were investigated under two conditions: a 12-hour daily photoperiod (light) and continuous darkness with 25/15 °C day/night temperature fluctuations (Singh & Singh, 2009). Petri dishes are coated with two layers of aluminum foil for incubation under dark conditions (Baskin & Baskin, 2014). The germination of the light treatment was monitored daily, and after 14 days of incubation, the final germination percentage for both the dark and light treatments was calculated.

pH

One of the necessary conditions for seeds to germinate in natural environments is the appropriate pH level. Seed germination, as influenced by pH, was evaluated using buffer solutions of pH from 4 to 10 prepared according to the method described by Chachalis & Reddy (2000). Pure water was used to adjust the pH. The acidity and alkalinity levels of the solution were adjusted by adding hydrogen chloride and sodium hydroxide to the pure water.

The impact of pH on seed germination was tested using buffer solutions of 4, 5, 6, 7, 8, 9, and 10 pH, which were measured by pH/mV desktop meter, Basic, equipped by mrc company, UK. After 14 days of maintaining Petri dishes with seeds at an alternating temperature of 25/15 °C, the germination percentage was determined.

Salinity

Seed germination as influenced by salt stress was evaluated using sodium chloride (NaCl) solutions of 0, 25, 50, 100, 150, and 200, and 250 mM. Seeds were placed in Petri dishes with 5-mL solutions of 0, 25, 50, 100, 150, 200, and 250 mM NaCl, which are produced by dissolving 0.375, 0.6, 1.45, 2.2, 2.925, and 3.65- g of NaCl in 250 mL of distilled water, respectively. After 14 days of incubation at different temperatures of 25/15 °C, the germination percentage is determined.

Measurement

Seed germination percentage (%): The proportion of seeds that germinate was estimated using the following equation:

$$Percent\ germination\ (\%) = \frac{Seeds\ germinated}{total\ seeds} * 100$$

Statistical Analysis

The experiments were conducted in a completely randomized design. All experiments were conducted with three replicates. The data were subjected to analysis of variance (ANOVA) using SPSS-22 software (SPSS In., Chicago, IL., USA), and means were separated using Fisher's LSD test at 0.05 probability. Statistical significance was determined using a P value of less than 0.05.

Results

Effect of temperature on seed germination

The influence of a constant temperature range for varied incubation periods on seed germination of *Lolium temulentum* L. is shown in Table 1. According to the results of the statistical analysis at the end of the experiment, the effect of different temperatures on the germination of *L. temulentum* seeds was found to be statistically significant. This weed's seeds did not germinate after one day at any of the temperatures examined, nor did they germinate at 35 °C and 40 °C for any period studied. After three days, the

first germination was recorded at temperatures of 20 °C, 25 °C, and 30 °C, with germination percentages of 3.33, 13.33, and 1.66 %, respectively. Seeds germinated by 40 % at 15 °C after 7 days, while at a temperature of 10 °C, seeds germinated after 14 days by 91.66% and at 5 °C after 28 days 90.83 %. The maximum germination percentage was 91.66 %, recorded at 10 and 20 °C after 14 days and stayed constant after 28 days. From the results, it was noted that the percentage of germination increases gradually with the length of the incubation period (Table 1).

Table 1. Effect of a constant temperature range for varied incubation periods on seed germination percentage of *Lolium temulentum* L.

Period	Temperature (°C)							
	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
1 day	0	0	0	0	0	0	0	0
3 day	0	0	0	3.33	13.33	1.66	0	0
7 day	0	0	40	59.16	44.16	5	0	0
14 day	0	91.66	90.83	91.66	84.16	79.16	0	0
28 day	90.83 a	91.66 a	90.83 a	91.66 a	84.16 b	79.16 c	0 d	0 d

*Treatments with the same letter are not statistically different; P ≤ 0.05

In the germination temperature study for *L. temulentum* seeds, it was determined that the most suitable germination was between 5-20 °C (Table 1 and 2). In the study, it is seen that 35 °C and above stops the seed viability activity. The germination temperature with the highest rate of germination (91,66 %) was determined as 20 °C. However, considering the total germination rates at the end of the experiment, there was no statistical difference between 5, 10, 15 and 20 °C, and they were all in the same group.

Looking at the germination times (T_{10}, T_{25}, T_{50} and T_{90}) of *L. temulentum* in the different temperature, it was determined that the seeds germinated in a minimum of 3 and a maximum of 28 days (Table 2). At temperatures outside of 5 °C, the seeds completed their germination in the first 14 days, and germination continued until the 28th day at 5 °C.

Effect of light on seed germination

The seed of *L. temulentum* did not germinate when incubated in darkness for 14 days. In contrast, it germinated at 32.5 % when incubated in light for 12 hours daily, as shown in Table 3 These findings suggest that seeds of this species may not germinate at greater soil depths. The germination of a seed is affected by light.

Table 3. Effect of lighting condition on seed germination of *Lolium temulentum* L.

Lighting condition	Seed germination (%)
Light	32.5 a
Dark	0 b

*Treatments with the same letter are not statistically different; P ≤ 0.05

Effect of pH on seed germination

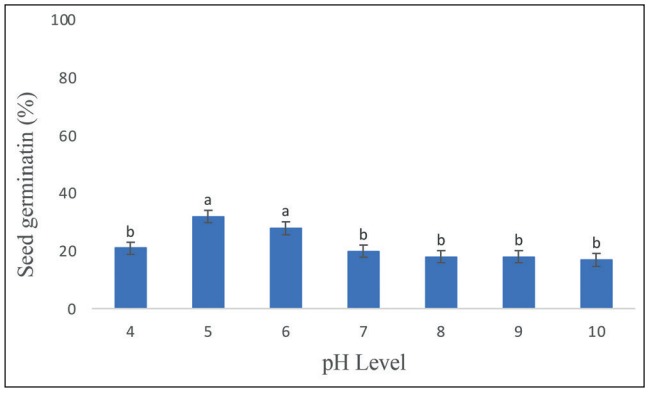
The findings of this study show that there is no significant influence of pH on seed germination of *L. temulentum* at any of the tested levels (Figure 1). This weed has no clear preference for a particular pH level, as shown by the fact that it germinates well throughout the pH range of 4 to 10. Although the highest germination rate is observed at pH 5, there is no statistically significant difference in germination rate between pH5 and pH6 levels (Figure 1). Most weeds have generalist features that enable them to grow in many soil types and habitats, including disturbed and degraded environments.

Table 2. Germination rates and durations of *Lolium temulentum* L. at different temperatures.

Temperature(°C)	G_{max} (%)	T_{10} (day)	T_{25} (day)	T_{50} (day)	T_{90} (day)
5 °C	91.11±1.1b*	28±0a	28±0a	28±0a	28±0a
10 °C	92.22±1.1a	14±0b	14±0b	14±0b	14±0b
15 °C	91.11±1.1b	7±0d	7±0c	14±0b	14±0b
20 °C	87.78±1.1c	7±0d	7±0c	7±0c	14±0b
25 °C	71.11±1.1e	3±0e	7±0c	7±0c	14±0b
30 °C	75.56±2.9d	11.67±2.3c	14±0b	14±0b	14±0b
35 °C	0±0f	0±0f	0±0d	0±0d	0±0c
40 °C	0±0f	0±0f	0±0d	0±0d	0±0c

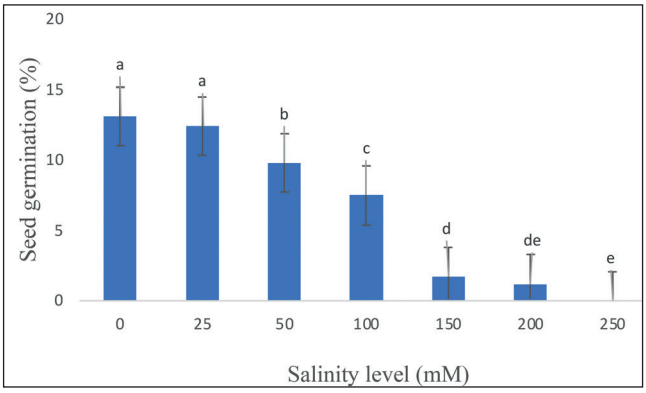
± = Standard deviation values. *Treatments with the same letter are not statistically different; P ≤ 0.05

Figure 1. Effect of different levels of pH on seed germination of *Lolium temulentum* L.



*Treatments with the same letter are not statistically different; P ≤ 0.05

Figure 2. Effect of different salinity levels on seed germination of *Lolium temulentum* L.



*Treatments with the same letter are not statistically different; P ≤ 0.05

Effect of salinity on seed germination

Data illustrated in Figure 2 showed significant effect of examined salinity levels on the seed germination of *L. temulentum*. Although the seeds of this weed did not germinate at a salinity level of 250 mM, the seed germination in the control treatment was 13.33 % (Figure 2).

Discussion

The findings of this study indicated that *Lolium temulentum* L. had the highest germination rate (91.66 %) when incubated at 10 °C and 20 °C for 14 days and that this percentage remained constant even after extending the incubation time. Seeds of this species may also germinate at low temperatures if the incubation period is prolonged. The seeds of this weed, on the other hand, did not germinate at temperatures of 35 °C or above, even after the longest incubation time tested, indicating that germination may be delayed, or the seed may become dormant at elevated temperatures. In the study conducted by Lu et al., (2006), the germination of Crofton weed (*Eupatorium adenophorum* Spreng.) seeds was examined by applying different factors. Although the seeds germinated at temperatures between 10 °C and 30 °C applied to the seeds, the optimum germination value was obtained at 25 °C.

Temperature is the most critical factor influencing germination, as it affects germination in three ways: moisture, hormone production, and enzyme activity (Finch-Savage & Leubner-Metzger, 2006). Seed germination needs a specific quantity of moisture; in a hot climate, moisture levels may decline, impacting germination (Baskin & Baskin, 2014). Temperature has an important effect on the weed species adaptability. Temperature of soil is a main ecological aspect that influences weed seed germination and development. The emergence rate of weeds is strictly associated with temperatures of soil (Singh & Singh., 2009). Temperature adjusts germination by eliminating dormancy (Benech-Arnold et al., 1990).

L. temulentum seeds did not germinate when incubated in darkness for 14 days. In contrast, it germinated at 32.5 % when incubated in light for 12 hours daily. Positively photoblastic seeds are driven to germinate by light, whereas negatively photoblastic seeds are those that are inhibited by light. The phytochrome-regulated synthesis of the plant hormone gibberellin modulates the response to light (Baskin & Baskin, 2014). The seeds were positively photoblastic, meaning that light encouraged germination while darkness inhibited it (Chen et al., 2013).

According to result of these experiments seeds of *L. temulentum* germinate across a wide pH range, indicating that this species is pH-tolerant and may survive in various soils. Florentine et al. (2016), Hao et al. (2017) and Humphries et al. (2018), who investigated diverse weed species, found comparable results. Furthermore, Perez-Fernandez et al. (2006) suggested that the germination processes in this species are not pH-dependent. Since Large Crabgrass (*Digitaria sanguinalis* (L.) Scop.) is a common weed in the fields, the effect of soil pH on Large Crabgrass was examined by Pierce et al., (1999). The seeds were planted in a loamy sand soil amended with calcium carbonate ($CaCO_3$) or magnesium carbonate ($MgCO_3$), which creates a soil pH range of 4.8 to 7.8. It was observed that the germination of Large Crabgrass seeds was not affected by the pH change when the soil was changed with $CaCO_3$, while it was observed that the seed germination decreased with the increasing pH value when it was replaced with $MgCO_3$. In another study conducted by Lu et al., (2006), the pH value of Crofton weed (*E. adenophorum*) germination was found between 5 and 7.

Singh & Singh (2009) studies were conducted on the effect pH and light exposure on seed germination of Brazil pusley, common ragweed, Florida beggarweed, hairy beggarticks, ivyleaf morningglory, Johnson grass, prickly sida, redroot pigweed, sicklepod, strangler vine, tall morningglory and yellow nutsedge. In the study, it was observed by the examiners that the species in terms of pH, it was observed that the pH range of 5 to 11 did not adversely affect the germination of weed species.

The change in soil pH level also changes the weed control efficiency. Chadha et al. (2019) stated that the decrease in control of *Abutilon theophrasti* Medicus and *Setaria faberi* Herrm. decreased when pH was raised from 5 to 6; however, control of *Amaranthus retroflexus* L. *and Chenopodium album* L. only occurred at the highest soil pH tested.

Outcomes of these research enounce that *L. temulentum* seeds could germinate in high saline circumstances, which can be an important issue for weed species allowing it to settle saline areas. Salinity is a significant abiotic stress factor for crop production globally. Soils with more than 100 mM NaCl are evaluated to have high salt substances. Crop manufacture might be affected through the soil salinity in addition to weed competition. Like *L. temulentum, Hibiscus tridactylites* Lindley (Chauhan, 2016) and *Mimosa invisa* Mart. ex Colla (Chauhan & Johnson, 2008) seeds germinated at very high salt concentrations. In another study conducted by Lu et al., (2006) on Crofton weed (*E. adenophorum*), the germination status of the weed changes at different salt concentrations applied. Crofton weed seeds germinated at values below 100 mM NaCl, but not at 300 mM NaCl. Swallowwort (*Cynanchum acutum* L.) seeds, another weed applied with different salt concentrations, did not germinate at 300 mM NaCl, but 12% germination was observed at 200 mM NaCl (Pahlevani et al., 2008).

High salinity may affect embryo viability primarily through hormonal changes, particularly abscisic acid (ABA) synthesis, which is known to induce or maintain seed dormancy, resulting in a lower germination rate and a longer germination time, or through other effects such as reduced water absorption and cell damage (Thiam et al., 2013; Ibrahim, 2016). The findings of this study confirm that the seeds of this species are salinity tolerant since they germinate even a small percentage at an extremely high salinity level (200 mM).

Conclusion

Weeds are found ubiquitously in almost every environment globally, and an important agricultural weed, *Lolium temulentum* L., grew and was challenging to manage in different climates conditions. Therefore, it is a weed that must be managed. In herbology studies carried out worldwide, weed control is generally carried out by looking at the biology and physiology of weeds. The study was carried out to understand how weeds respond to light, temperature, pH, salinity and when these germination processes are activated throughout the stress.

According to the findings of this study, the optimum germination temperature for seeds of this species is between 10 and 20 degrees Celsius, and if there are temperatures of 20 °C and above, they can start to germinate within 3 days. Seeds of this species take 28 days to germinate at temperatures around 5 °C, and cold weather slows germination. According to the study, the seeds of this species were also positively photoblastic, indicating that they may not germinate in darkness. Furthermore, there was no influence of pH on the weed's seed germination, showing that it may germinate and grow in a range of soils. According to seed germination studies, this species is salt resistant and can tolerate salinity of up to 200 mM NaCl.

The data obtained from the study show that *L. temulentum* can germinate and spread even in extreme environments such as temperature, pH and salinity. This shows that this species has the potential to spread rapidly and cause damage in agricultural areas. Therefore, it is necessary to take measures to prevent the spread of this species. In preventing the spread of *L. temulentum*, clean production materials and equipment to be used in the field are clean, animal manures that may contaminate weed seeds are not used, if irrigation is to be done, attention should be paid not to let weed seeds come to the field with irrigation water, and plant rotation is among the important issues.

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TEMPORAL CHANGES OF VASCULAR PLANT DIVERSITY IN RESPONSE TO TREE DIEBACK IN A MEDITERRANEAN LOWLAND FOREST

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Abstract – Lowland forests underwent a century long history of deforestation and degradation that left only few remnants of this forest type, especially in the Mediterranean region. These remnants have high conservation value but are threatened by tree diebacks related to different causes. Here we focused on the area of Palo Laziale (oak floodplain forest) and on the effects of the tree dieback following the summer drought of 2003. In the framework of an ecological restoration project (LIFE PRIMED LIFE17 NAT/GR/000511), we collected data on plant communities' species composition both in 2019/2020 and compared these data to those collected in 1990, also accounting for life forms, chorotypes and Ellenberg Indicator Values. This analysis was conducted to assess whether there was any change in the species composition following the forest dieback. The total flora of the site increased from 462 to 490 species. Moreover, there has been a turnover of species with the loss of some grassland and halophytic species and the entrance of allochthonous/ruderal and freshwater habitat species. Despite this, the plant diversity remained unchanged in bioindication terms, demonstrating a certain resilience of the ecosystem plant species, confirming the floristic re-survey approach to identify declining processes and support ecosystem-based restoration actions elsewhere.

Keywords: ecological restoration, EU habitats, flora analysis, LIFE programme, Natura2000.

Introduction

Coastal lowland forests and transitional wetlands are among the EU's most degraded and threatened ecosystems (Britton & Crivelli, 1993). They host a high level of biodiversity, especially for vascular plants and invertebrates and can provide many ecosystem services (Bonan, 2008; Nocentini et al., 2022). In particular, these ecosystems, are a sink for carbon and their degradation could reduce their potential to sequester it (Breshears & Allen, 2002). Furthermore, they are clearly defined from a physiognomical, geomorphological and ecological point of view (Chytrý et al., 2020).
Due to the flat morphology, these areas are suitable for human activities, which menace their biodiversity. The main anthropogenic pressures are infrastructure development, land conversion, drainage works, pollution, overexploitation, and invasive alien species (Millennium Ecosystem Assessment, 2005; Díaz et al., 2019). This, in turn, increases their vulnerability to climate change (extreme weather events, topsoil aridity, uneven rainfall regimes) which is also very impactful (De Dios et al., 2007). Many Mediterranean lowland forest of Italy can be found under these conditions, and they host several habitats and species with an 'unfavourable', 'vulnerable' or 'near-threatened' conservation status according to the Habitats Directive (Ercole et al., 2021).
There are several zones with residual lowland forests in the Italian Peninsula, such as the Po Valley, Policoro plain and the Tyrrhenian Coast. Until a few centuries ago, the latter was an expanse of marshland and forest, which has been intensively fragmented over time. These areas have been reclaimed

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and subsequently cultivated or built upon, leading to an inexorable loss of biodiversity (Lucchese & Pignatti, 1990). Some of the remaining patches of lowland forest have been preserved because they were noble hunting grounds, (e.g. Castel Porziano natural reserve) although some of them have over time been fragmented and/or surrounded by industry, farmland, and urban areas.

Another threat of the Mediterranean lowland forest is represented by oak forest dieback, which is increasing dramatically in different forest ecosystems (Allen, 2009; Colangelo et al., 2017; Maselli, 2004; Ogaya et al., 2015). It is mainly caused by prolonged droughts, sudden flooding and rapid fluctuations in soil water levels (Brasier, 1996; Gutschick & BassiriRad, 2003; Levanič et al., 2011). Individually, none of these phenomena is responsible for the forest decline, but all of them cause undue stress to the oak trees and lay the groundwork for secondary attacks by saproxylic insects (e.g. *Agrilus* spp., *Scolytus* spp.) and increased susceptibility to opportunistic pathogenic fungi of the stems, leaves and roots of the plants (*Discula quercina*, *Diplodia corticola*, *Armillaria* spp., etc.).

In Italy, oak woodlands, from north to south of the Peninsula, have also been subject to decline, reduction, and death of forest stands under a wide range of environmental conditions (Bertini et al., 2011; Di Filippo et al., 2010, Conte et al., 2019). The Palo Laziale wood is one such case, with the first symptoms of forest dieback appearing in 1995 and gradually exacerbating until the arid summer of 2003 when about 40% of the adult trees were found dead (Scarnati & Attorre, 2014).

To address the forest dieback of Palo Laziale, a Nature and Biodiversity LIFE project, LIFE PRIMED LIFE17 NAT/GR/000511 "Restoration, management and valorisation of PRIority habitats of MEDiterranean coastal areas)" was started in 2018. It is an interdisciplinary project aiming at improving the conservation status of the habitats and species of Nestos Delta and Palo Laziale Wood Natura 2000 sites in Greece and Italy, respectively.

Palo Laziale preserves a high biological diversity due to several habitats becoming progressively rare in the lowland areas of Lazio (Della Bella et al., 2005; Fraticelli & Sarrocco, 2012; Pizzuti Piccoli, 2016). The plant diversity is well documented by a study (Lucchese, 1990) highlighting its remarkable diversity and providing a snapshot of the environmental situation before the forest dieback. Changes in plant species diversity go far beyond the description of taxonomic composition if viewed from the perspective of plants as ecological indicators (Pignatti et al., 2001). It gives proxies of the interrelationships among components of the ecosystem, that are very difficult to gain with other approaches. Notwithstanding habitat restoration initiatives are increasingly gaining momentum (Wortley et al., 2013), detecting changes in plant checklists biodiversity remains a kick-off practice relatively unexplored by ecologists and practitioners. However, a large network of European botanists acknowledging the importance of plant taxa, are starting to create a database that will allow in the future to assess the importance of diachronic analyses (see ReSurveyEurope initiative)

Forest dieback could have effects that go far beyond the composition and structure of forests alone. A catastrophic event such as the one observed in Palo Laziale could affect the entire ecosystem in all its components, both arboreal and herbaceous. In order to assess any ecosystem changes, it is necessary to consider the entire plant diversity, both forest, aquatic and grassland habitats, so as to ascertain how the entire vegetal landscape may have changed. For this purpose, the diachronic study of the plant taxa, i.e. the comparison of the species present before and after dieback, is irreplaceable since the only historic data available is a plant checklist.

For this study, we used the valuable species list from Lucchese (1990), a high-quality work rarely available in the areas studied. This data is not as rich as a detailed phytosociological study of the vegetation and forest structure, but these data prior to dieback were unavailable. The aim is to emphasise the importance of the diachronic analysis of plants when dealing with the conservation and management of declined ecosystems repeating the floristic sampling 30 years after the first time to identify and quantify overall changes and effects of the forest dieback on the plant diversity of the site.

Materials and Methods

Study area

The Palo Laziale Wood is one of the last remaining patches of lowland forest along the coast of the Lazio Region that once covered the shoreline from the Tiber mouth to Capo Linaro (Barca et al., 1981; Fraticelli et al., 1995). It is located about 40 km northwest of Rome, directly facing the Tyrrhenian Sea. It is a flat area of about 130 hectares, with an altitude between 3 and 10 metres above sea level.

The woodland area is located within a private property, entirely fenced in, and part of the SAC IT6030022 Bosco di Palo Laziale.

The predominant vegetation of the area is represented by a deciduous forest, with a prevalence of *Quercus cerris* and rare individuals of *Q. petraea* and *Q. frainetto* (*Crataego laevigatae-Quercion cerridis* Arrigoni 1997). In drier and warmer areas, Mediterranean sclerophyllous scrub can be found with a predominance of *Quercus ilex*, *Pistacia lentiscus* and *Phillyrea*

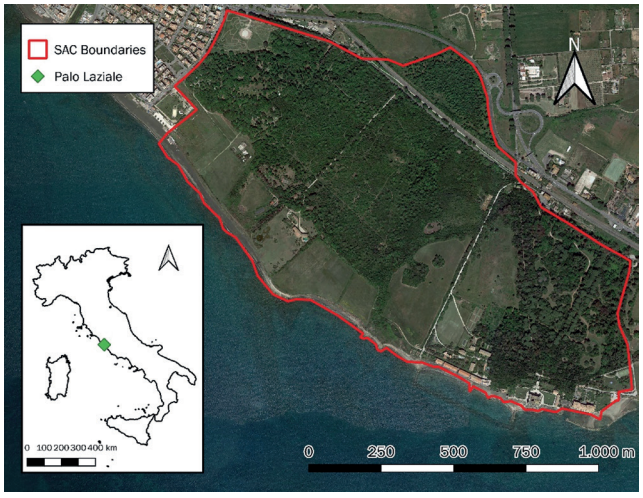

Figure 1. Map of the study site with the SAC boundaries.

latifolia (*Quercion ilicis* Br.-Bl. ex Molinier 1934). One of the peculiarities of this forest is that during periods of heavy rainfall, it becomes completely swamped, with pools in the depressed areas persisting until late summer and the vegetation changes drastically, with *Fraxinus angustifolia* subsp. *oxycarpa* and *Quercus robur* (*Lauro nobilis-Fraxinion angustifoliae* Kárpáti I. & Kárpáti V., 1961). For the syntaxonomy, refer to Mucina et al. (2016). There are also numerous open areas with dry and wet herbaceous vegetation ranging in size from a few square meters to a few hectares.

The Palo Laziale woodland occurs in an area with almost exclusively Plio-Quaternary deposits (ISPRA, 2014). The study site's littoral is included in the lower Mesomediterranean thermotype and the upper dry/lower sub-humid umbrotype (Blasi, 1994).

The intensity of the forest dieback was quite high. The first signs were observed in 1995, then in 2003 approximately 40% of trees individuals died due to the high heat waves of those years and as a consequence of the fungal pathogen attack of *Biscougnaxia mediterranea*. Moreover, in 2004, to prevent the spread of this pathogen, phytosanitary clear-cuts were performed in the area (Scarnati & Attorre, 2014).

Sampling methods

The fieldwork was carried out both in 2019-2020 to compile a list of vascular plant species.

The sampling protocol consists of exploring exactly the same area investigated by Lucchese (1990), noting all the plant species present in all seasons of the year, approximately once a week in the flowering season (March-June) and approximately every 15 days in the dormant period. No plots were recorded as the aim was to compare the current species list with the 1990's list. During sampling, voucher specimens were collected to check identifications.

The material collected is deposited in the Fanelli collection of the Rome Herbarium, Italy (Herbarium Code: RO). Pignatti et al. (2017) and various monographs were used for identifications. For the *Isoetaceae*, Troìa and Greuter (2015) was consulted, while for *Bolboschoenus* spp. we followed Hroudová et al. (2007) and for *Viola* subsect. *Viola,* Hodálová et al. (2008) was used. The nomenclature follows Bartolucci et al. (2018). Afterwards, the nomenclatural alignment between the lists, two species from the 1990's list were synonyms, but it did not affect significatively the species counting.

Data analysis

A comparative analysis was conducted between our checklist and that of Lucchese (1990). Turnover was calculated using the Sørensen index (Sørensen, 1948) which is recommended for presence-absence data (Vellend, 2001). Calculation of dissimilarity between the two lists, were calculated using "vegan" package in R (Oksanen et al., 2013).

A number of indicators were used to compare the two lists. In particular, the percentages of the biological forms, chorotypes and Ellenberg indices were calculated. The chorotypes represent the geographical distribution patterns of the species and are taken from Pignatti (2005), aggregating them into the main forms. Further, Raunkiær's biological forms were used, again taken from Pignatti (2005).

Ellenberg indicator values (EIVs) are a series of 6 numbers (L light, T temperature, K continentality, F moisture, N nutrient, R pH, S salinity) representing the factors that determine the typical environmental conditions of the species. They were applied to all the species list. The values were taken from Fanelli et al. (2007) and Pignatti et al. (2001) for the native flora and from Domina et al. (2018) for the alien flora.

For the ecological characterisation, a seventh value was added, the hemeroby value, which can give the idea of the disturbance level and the influence of anthropic impact (Hill et al., 2002; Kowarik, 1999). The recently published list in Midolo et al. (2023) was not considered because many species from our list are missing. Finally, the species list was divided into native and alien species.

To compare the two lists, visual analysis was first carried out by graphing the percentages of the various indicators. Barplots were used for the chorological types and biological forms. While for the EIVs and hemeroby value, multiple line plots were made.

To test which parameters or indicators between the 1990 list and ours are significantly different, G-test (Signorell et al., 2019) was used. All analyses were performed with the R software.

The whole set of raw data is available in Supplementary Table S1.

Results

We found 490 plant taxa, a number higher than that reported in the 1990 checklist (462 taxa).
The turnover of species and subspecies was massive in the last 30 years, with 146 taxa newly found, 116 taxa no longer found from the old checklist, and 344 species in common (fig. 2). From the comparison between alien and native species, the G-test highlighted a significant difference between newly and no longer found species ($p = 0.049093$). Sörensen dissimilarity index is 0.272, indicating a substantial change of the flora over 30 years.
According to the chorological spectra, a few differences between the two surveys can be observed (fig. 3). Cosmopolitan and Eurasiatic species have slightly decreased, while Eurimediterranean have slightly increased. There is also an increase in naturalised adventitious plants, including many allochthonous species, some of which invasive. The G-test indicates that none of the variations are significant ($p > 0.05$).
Looking at the biological spectra (fig. 3), an increase in the number of therophytes, phanerophytes, nano-phanerophytes, hydrophytes and chamaephytes was observed together with a decrease of geophytes and hemicryptophytes, with no significative differences.

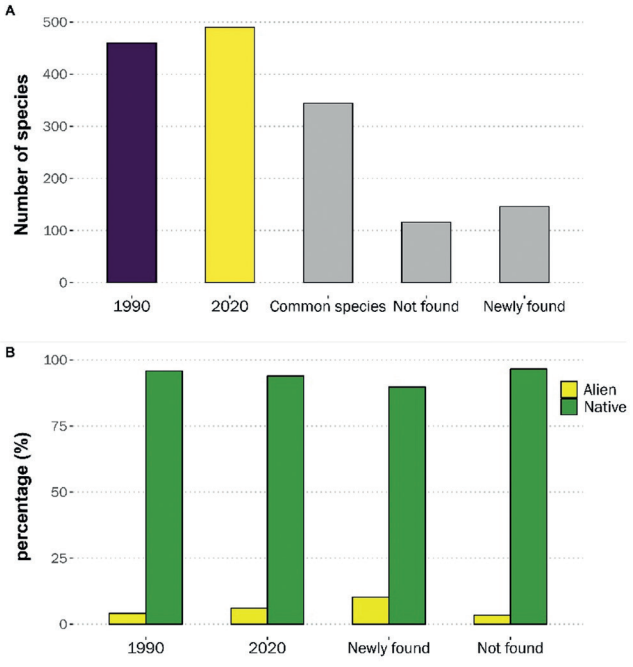

Figure 2. Comparison of floristic data from Lucchese (1990) and 2020 data. A) Total species number and number of common, newly and no longer found species above. B) Percentage of alien and native species. According to the G-test, the only significative difference is between the newly and no longer found species on the percentage of alien species ($p < 0.05$).

EIVs showed a reduction in the light (L), continentality (K) and salinity (S) values in 2020. All other indicators are stable, except for the nutrient value (N), which increased slightly. None of these differences are significant (fig.4).
If we focus on the EIVs calculated for the turnover species, i.e., no longer found species from the old checklist and the newly found species in ours, we notice some interesting results. The species previously present required high values for luminosity (L), continentality (K), salinity (S) and hemeroby (H). In contrast, the species that have colonized the area in the last 30 years tend to have lower values, thus explaining the decrease in the values of the ecological indicators for the general plant species. In particular, the hemeroby value is the only one with significant differences between the two years ($p = 0.04603$). Among the species changed, it can be seen lower hemeroby values for categories 0-2, and an increase of higher-intermediate hemeroby values (category 6). Higher category of hemeroby values (categories 7-9) shows an increase in species for 2020 and a decrease for 1990 species. On the other hand, species of categories 3-4 increase in the newly found species (fig.5).

Discussion and Conclusion

The approach used in this work follows the theory of the plants as ecological bioindicator herited from Sandro Pignatti (Pignatti et al., 2001). This approach is very powerful, and it can help to understand the interaction among species. Unfortunately, little is known and understood by a broader audience. In this paper, we tried to use this approach to address the problem of a very highly biodiverse habitat that suffered a dramatic collapse a few years ago.
Dieback events are becoming more frequent in the Mediterranean, affecting different species of trees (Rozas & Sampedro, 2013; Touhami et al., 2020). They have been related to various environmental phenomena, particularly periods of severe drought. Diebacks are also often associated with parasite outbreaks, especially fungi (Sallé & Bouget, 2020; Thomas, 2008). Palo Laziale dieback fits perfectly into the general pattern, as it coincides with the driest year of the last 20 years and the outbreak of certain non-pathogenic fungal species that become aggressive under these stressful conditions (Beccaccioli et al., 2021; Mazzaglia et al., 2001). This phenomenon seems to be becoming increasingly frequent in connection with global change (Allen, 2009).
While the drivers of dieback are the subject of intense investigation (McDowell et al., 2022), studies investigating the effect of such catastrophic events on the ecosystem as a whole are much rarer or non-existent. In this study, we were fortunate to compare the flora before and after the severe

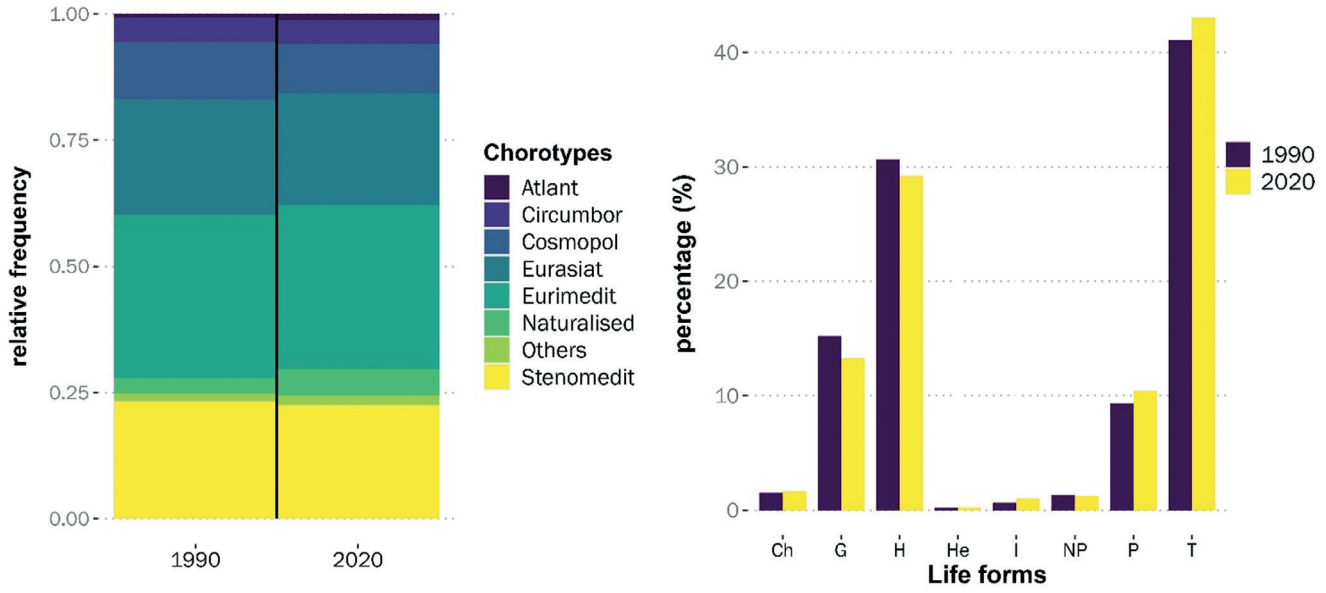

Figure 3. Differences between chorotypes and life forms of the two floristic data studied. Some chorotypes have been grouped into the category "Others" (Endemics + South European Orophytes + Mediterranean Mountain). The differences were tested with G-test showing no significative differences ($p > 0.05$).

case of dieback of 2003, using this information as a proxy for the effects on the ecosystem.

The total number of species in the Palo Laziale wood has remained unchanged over the last three decades. Some rare species have disappeared (*Carex grioletii, Juncus gerardii*) due to an alteration of the habitat conditions (forest disturbance and dilution of saltmarsh substrates, respectively) but, on the other hand, species of equal conservation interest have appeared (*Solenopsis laurentia*). The case of *Carex grioletii* is ecologically interesting because it is a microtherm species and an indicator of relict vegetation with little anthropisation, and where it tends to disappear it is replaced by its congener *Carex sylvatica* with similar ecological characteristics but much more adapted to disturbance (Montelucci, 1952).

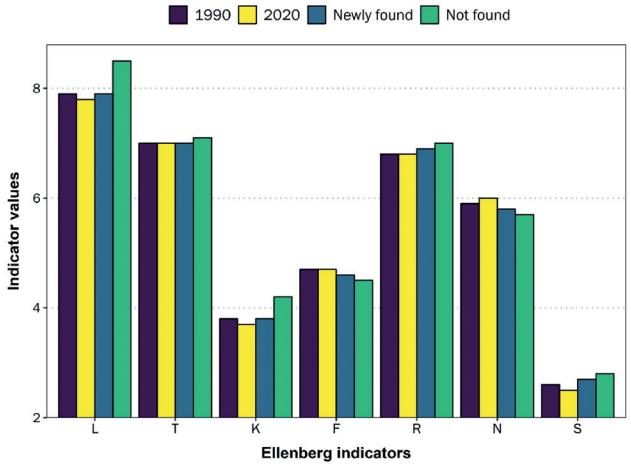

Figure 4. Ellenberg Indicator Values (EIVs) in comparison between the two years and the turnover species. There are some differences between the data, but the G-test showed they are not significant ($p > 0.05$).

Although the richness of the flora has remained unchanged, there has been a noticeable turnover in species. The turnover of flora is relatively high (dissimilarity = 0.272). No longer found species belong to two main groups: grassland and halophilous species. Although the meadows of Palo Laziale are still remarkably rich in species, some species typical of Mediterranean perennial meadows have not been found (*Brachypodium phoenicoides*, *Anacamptis morio*, *Medicago orbicularis*, etc).

Analysis of the indicators shows that although the composition has changed qualitatively, it does not change regarding chorological, biological and ecological groups. Salinity (S) decreases slightly, and it is quite challenging to explain this variation, but it could be related to changes in the water table level. Interestingly, the number of thermophyte species remains unchanged even though the climatic trends of the area show a conspicuous increase in temperatures over the past thirty years. These results are only qualitative but suggest a trend likely to become significant in the future. The finding of wetlands species often of high conservation value is worth mentioning, such as *Solenopsis laurentia*, *Isolepis cernua*, *Epilobium tetragonum*, etc. These species seem to indicate an increase in humidity.

While the composition of the flora and the number of species remain unchanged before and after the dieback, a significant change is an increase in the number of allochthonous

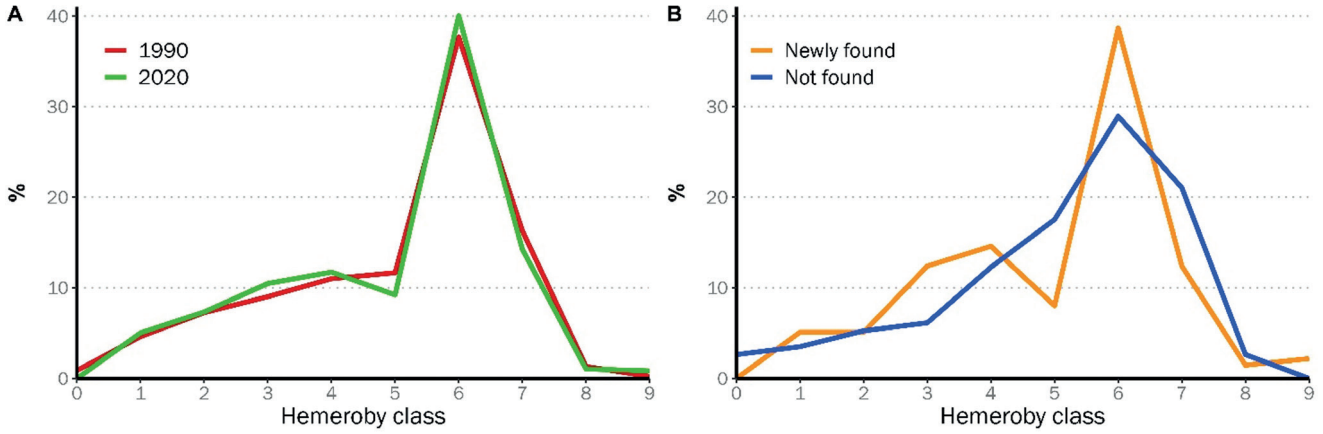

Figure 5. Hemeroby indicator values in comparison between: A) the two years (1990, 2020) and B) between the newly and no longer found species. They were tested with G-test showing no significance between two years datasets ($p > 0.05$), while on the other hand, the turnover species showed a significant difference ($p < 0.05$).

(comparing no longer found and newly found species). Numerous allochthonous species have been found, some of which are invasive (*Cortaderia selloana*, *Araujia sericifera*, *Lonicera japonica*, etc.). This trend is consistent with what has been observed in Rome, more or less in the same period (Fratarcangeli et al., 2022). The expansion of invasive species is a global phenomenon and represents one of the greatest threats to biodiversity (Rosenzweig, 2001). This expansion is not fully understood. Some authors relate it to the increase in propagule pressure resulting from increased human communication and traffic (Van Kleunen et al., 2015). On the other hand, the presence of allochthonous species is often observed in highly disturbed environments (Garzia et al., 2019; Haeuser et al., 2017). In Palo Laziale, the increase in allochthonous species seems to be related to the increase in disturbance, as evidenced by the change in the Hemeroby index (fig.5). Many changes occurred between 1990 and 2020 (phytosanitary cuts, excavation of temporary pools, urban development in the surrounding areas), but the most intense disturbance during this period seems to be the dieback. Indeed, in addition to being a threat to forest communities, it represents a catastrophic phenomenon that may have altered the entire ecosystem. In fact, dieback opened gaps in the vegetation and increased the amount of dead wood and changed the cycle of nutrients in the soil, causing the ingression of species previously non-existent in the ecosystem, such as fringe species and aliens. This effect is poorly documented in the literature (but see Devagiri et al., 2016) and is commonly observed in field surveys.

Disentangling the stress factors and disturbance effects in a complex natural ecosystem is challenging. The analysis of the temporal changes in flora diversity allows us to retrace past stress events and verify the effects of anthropogenic and non-anthropogenic pressures on threatened habitats to help prevent inappropriate management measures (e.g., excessive digging of salt ponds). Such an approach can provide a compelling ecological indication with a relatively low effort to support focused restoration practices. This work showed remarkable plant diversity and remarkable stability in the number of plant species despite the strong disturbance that intervened between the two censuses. Considering that plant species are indicators of the state of ecosystems (Pignatti et al., 2001) this suggests that the ecosystem of Palo Laziale has a high resilience. The ultimate goal of the current LIFE project is to maintain this kind of response favouring the ecological conditions which enhance the floristic composition, in order to counteract future tightening of climate regimes and human impacts.

The site of Palo Laziale well-represents the typical heterogeneity and richness of the Mediterranean natural mosaics, although its small extent may exacerbate the effects of external sources of disturbance. (Rösch et al., 2015). To maintain such a remarkable level of habitat and species diversity, is important to keep the resilience of the ecosystems equally high (Timpane-Padgham et al., 2017). Species turnover would become unavoidable in quickly changing environments (Brown, 1995; van der Maarel & Sykes, 1993). A stable number of species could secure optimal occupancy of the ecological roles in functional and healthy communities (Ferlian et al., 2018). Decades of biodiversity-ecosystem functions research has provided compelling evidence for a largely positive relationship between biodiversity and ecosystem functioning in most cases (Cardinale et al., 2012). Ensuring the variability of abiotic and biotic factors, rather than passively conserving the existing categories of habitats, should be among the most appropriate management decision on a long-term basis for dynamic ecosystems. Monitoring is paramount, especially for unveiling local effects of large-scale phenomena like urbanisation, human disturbance, and climate change (Ceschin et al., 2010; Searcy, 2012; Wirth

et al., 2020). Comparing the changes in flora composition of Palo Laziale and elsewhere over time (see Cornelini & Petrella, 1996; Rich & Karran, 2006; Salinitro et al., 2019; Todini & Crosti, 2020) has proven to be a promising approach to identify declining processes and support ecosystem-based restoration actions elsewhere. The results of this work call for more integration of the diachronic studies of flora into conservation decision-making.

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Raw datasets

DATASET: "La Montagna et al 2023"

Species	Year	Chorotype	Main life form	L	T	K	F	R	N	S	H
Acacia_dealbata	2020	Naturalised	P	9	9	5	6	5	3	0	7
Acer_campestre	2020	Eurasiat	P	5	7	4	5	7	6	0	3
Acer_monspessulanum	2020	Eurimedit	P	6	8	5	3	8	4	0	1
Aegonychon_purpocoeruleum	2020	Eurasiat	H	5	7	6	4	8	4	0	1
Agave_americana	2020	Naturalised	P	9	10	2	2	X	2	0	1
Agrostis_stolonifera	2020	Circumbor	H	8	X	X	6	X	5	0	6
Aira_elegantissima_subsp_elegantissima	2020	Eurimedit	T	8	9	5	2	3	1	0	6
Alisma_plantago-aquatica	2020	Cosmopol	I	7	X	X	1	X	8	0	6
Alliaria_petiolata	2020	Eurasiat	H	5	6	5	5	7	9	0	2
Allium_neapolitanum	2020	Stenomedit	G	6	9	4	4	4	7	0	4
Allium_roseum_subsp_roseum	2020	Stenomedit	G	8	8	4	3	6	5	0	5
Allium_triquetrum	2020	Stenomedit	G	6	9	4	4	4	7	0	1
Alopecurus_myosuroides_subsp_myosuroides	2020	Cosmopol	T	6	6	5	6	7	7	0	6
Amaranthus_deflexus	2020	Naturalised	T	8	8	5	4	6	9	0	7
Amaranthus_retroflexus	2020	Naturalised	T	9	9	7	4	X	9	0	7
Anacamptis_laxiflora	2020	Eurimedit	G	8	7	5	6	6	5	0	6
Anacamptis_papilionacea	2020	Eurimedit	G	8	8	5	3	6	4	0	6
Anacyclus_radiatus_subsp_radiatus	2020	Stenomedit	T	8	9	4	4	5	2	0	7
Andryala_integrifolia	2020	Eurimedit	T	8	9	3	2	2	1	0	6
Anisantha_diandra	2020	Eurimedit	T	8	8	5	3	5	4	0	6
Anisantha_madritensis	2020	Eurimedit	T	8	7	5	3	X	1	0	6
Anisantha_rigida	2020	Cosmopol	T	8	8	5	4	6	5	0	6
Anisantha_sterilis	2020	Eurimedit	T	8	11	5	2	X	2	0	6
Anthemis_maritima_subsp_maritima	2020	Others	H	11	9	4	1	X	1	0	3
Anthoxanthum_odoratum	2020	Eurasiat	H	X	X	5	X	5	3	0	4
Araujia_sericifera	2020	Naturalised	T	9	9	5	3	5	5	0	8
Arbutus_unedo	2020	Stenomedit	P	11	9	4	3	4	2	0	2
Arisarum_vulgare_subsp_vulgare	2020	Stenomedit	G	6	8	4	4	4	4	0	3
Artemisia_absinthium	2020	Others	Ch	9	6	7	4	X	8	0	6
Arum_italicum_subsp_italicum	2020	Stenomedit	G	6	8	4	4	5	5	0	3
Arundo_plinii	2020	Stenomedit	G	11	8	4	4	4	2	0	4
Asparagus_acutifolius	2020	Stenomedit	G	6	9	4	2	5	5	0	2
Asperula_laevigata	2020	Stenomedit	H	6	6	4	4	7	3	0	2
Asphodelus_ramosus_subsp_ramosus	2020	Stenomedit	G	11	9	4	2	3	5	0	4
Asplenium_onopteris	2020	Cosmopol	H	3	9	4	3	5	3	0	1
Asplenium_trichomanes_subsp_quadrivalens	2020	Cosmopol	H	5	X	5	5	X	4	0	2
Atriplex_prostrata	2020	Circumbor	T	9	X	X	6	X	9	0	7
Avena_barbata	2020	Eurimedit	T	8	8	5	3	7	2	0	6
Avena_fatua	2020	Eurasiat	T	6	X	6	6	7	X	0	6
Avena_sterilis	2020	Eurimedit	T	8	9	5	3	6	4	0	6
Ballota_nigra_subsp_meridionalis	2020	Eurimedit	H	8	6	5	5	X	8	0	7
Barbarea_vulgaris	2020	Cosmopol	H	8	X	5	7	X	6	0	6
Bellardia_viscosa	2020	Eurimedit	T	8	8	3	3	3	3	0	6
Bellevalia_romana	2020	Eurimedit	G	8	7	5	3	6	4	0	6
Bellis_annua	2020	Stenomedit	T	6	9	4	7	2	2	0	7
Bellis_perennis	2020	Eurasiat	H	9	5	4	X	X	5	0	7
Bellis_sylvestris	2020	Stenomedit	H	5	8	4	3	3	3	0	3
Beta_vulgaris_subsp_vulgaris	2020	Eurimedit	H	11	7	5	6	6	5	1	6
Betonica_officinalis	2020	Eurasiat	H	6	5	4	6	4	3	0	2
Bidens_frondosa	2020	Naturalised	T	7	7	X	9	7	8	0	7
Blackstonia_perfoliata_subsp_perfoliata	2020	Eurimedit	T	8	7	5	X	9	4	0	4
Bolboschoenus_glaucus	2020	Eurasiat	G								
Borago_officinalis	2020	Eurimedit	T	7	8	5	3	5	5	0	6
Brachypodium_distachyon	2020	Stenomedit	T	11	9	3	1	3	2	0	4
Brachypodium_rupestre	2020	Atlant	H	8	6	4	5	8	4	0	4
Brachypodium_sylvaticum_subsp_sylvaticum	2020	Eurasiat	H	4	5	5	5	6	6	0	2
Briza_maxima	2020	Cosmopol	T	8	10	5	2	4	1	0	5
Briza_minor	2020	Cosmopol	T	8	9	5	2	4	1	0	6
Bromus_hordeaceus_subsp_hordeaceus	2020	Cosmopol	T	7	6	5	X	X	X	0	6
Bryonia_dioica	2020	Eurimedit	G	8	7	5	5	8	6	0	3
Bunias_erucago	2020	Eurimedit	T	8	8	5	4	5	3	0	6

Cakile_maritima_subsp_maritima	2020	Eurimedit	T	9	8	2	6	X	8	2	3
Calamagrostis_epigejos_subsp_epigejos	2020	Circumbor	H	12	6	5	4	7	5	2	6
Calendula_arvensis	2020	Eurimedit	T	7	8	5	3	8	5	0	6
Calepina_irregularis	2020	Eurimedit	T	8	8	4	3	5	3	0	6
Callitriche_stagnalis	2020	Eurasiat	I	9	8	5	12	5	1	0	5
Campanula_erinus	2020	Stenomedit	T	7	8	4	2	X	1	0	4
Campanula_rapunculus	2020	Eurasiat	H	7	7	5	4	6	4	0	6
Campsis_radicans	2020	Naturalised	P	9	7	5	5	5	4	0	9
Capsella_bursa-pastoris_subsp_bursa-pastoris	2020	Cosmopol	H	7	X	5	5	5	4	0	7
Capsella_rubella	2020	Eurimedit	T	8	9	5	2	4	2	0	7
Cardamine_hirsuta	2020	Cosmopol	T	7	8	5	3	5	4	0	6
Carduus_nutans_subsp_nutans	2020	Atlant	H	8	X	5	3	8	6	0	6
Carduus_pycnocephalus_subsp_pycnocephalus	2020	Eurimedit	H	7	8	4	3	X	3	0	6
Carex_depauperata	2020	Eurimedit	H	4	4	4	6	X	7	0	2
Carex_distachya	2020	Stenomedit	H	6	6	4	2	4	5	0	1
Carex_divisa	2020	Eurimedit	G	8	8	2	3	5	3	0	6
Carex_divulsa	2020	Eurimedit	H	7	6	5	4	5	5	0	5
Carex_flacca_subsp_erythrostachys	2020	Eurasiat	G	7	5	5	6	8	X	0	4
Carex_flacca_subsp_flacca	2020	Eurasiat	G	7	5	5	6	8	X	0	3
Carex_otrubae	2020	Eurimedit	H	9	5	5	9	X	5	0	7
Carex_punctata	2020	Eurimedit	H	7	6	3	10	4	3	0	3
Carex_spicata	2020	Eurasiat	H	7	6	5	4	5	5	0	7
Carex_sylvatica	2020	Eurasiat	H	2	5	3	5	7	5	0	2
Carlina_corymbosa	2020	Stenomedit	H	6	X	4	2	X	4	0	5
Carpobrotus_edulis	2020	Naturalised	Ch	9	10	4	1	X	1	1	2
Carthamus_caeruleus	2020	Eurimedit	H	11	11	5	3	7	4	0	5
Carthamus_lanatus	2020	Eurimedit	T	11	8	5	3	5	6	0	6
Catapodium_rigidum	2020	Eurimedit	T	8	8	5	2	5	4	0	6
Centaurea_jacea_subsp_gaudinii	2020	Eurasiat	H	6	5	7	4	7	3	0	6
Centaurea_napifolia	2020	Stenomedit	T	8	11	5	4	6	3	0	6
Centaurea_solstitialis	2020	Stenomedit	H	11	9	4	3	X	5	0	6
Centaurea_sphaerocephala_subsp_sphaerocephala	2020	Stenomedit	H	11	10	4	1	X	1	0	6
Centaurium_erythraea_subsp_erythraea	2020	Eurasiat	H	8	6	5	5	6	X	0	4
Centaurium_maritimum	2020	Stenomedit	T	11	9	4	3	3	1	0	5
Centaurium_pulchellum_subsp_pulchellum	2020	Eurasiat	T	9	6	7	7	9	3	0	4
Centaurium_tenuiflorum	2020	Eurasiat	T	9	8	5	7	7	2	0	4
Cephalaria_transsylvanica	2020	Eurasiat	T	7	6	7	3	7	2	0	4
Cerastium_brachypetalum	2020	Eurimedit	T	11	7	5	3	7	2	0	6
Cerastium_glomeratum	2020	Eurimedit	T	7	X	5	5	5	5	0	7
Cerastium_ligusticum	2020	Stenomedit	T	11	9	4	2	3	1	0	6
Cerinthe_major_subsp_major	2020	Stenomedit	T	7	8	4	4	5	9	0	6
Chamaeiris_foetidissima	2020	Eurimedit	G	7	7	5	4	4	5	0	2
Chamaemelum_fuscatum	2020	Others	T	8	8	4	3	3	2	0	6
Chamaerops_humilis	2020	Stenomedit	NP	11	10	3	1	4	1	0	1
Chenopodium_album	2020	Cosmopol	T	7	7	5	4	5	7	0	7
Cichorium_intybus	2020	Eurasiat	H	9	6	5	3	8	5	0	6
Cirsium_vulgare_subsp_vulgare	2020	Eurasiat	H	8	5	5	5	X	8	0	6
Clematis_vitalba	2020	Eurasiat	P	7	7	4	5	7	7	0	3
Clinopodium_nepeta	2020	Others	H	5	7	5	3	9	3	0	6
Clinopodium_vulgare_subsp_vulgare	2020	Circumbor	H	7	5	4	4	7	3	0	3
Coleostephus_myconis	2020	Stenomedit	T	8	9	4	3	5	4	0	6
Convolvulus_althaeoides	2020	Stenomedit	H	8	9	4	3	5	2	0	4
Convolvulus_arvensis	2020	Eurasiat	G	7	7	5	4	5	5	0	6
Convolvulus_sepium	2020	Eurasiat	H	8	6	5	6	7	9	0	7
Cortaderia_selloana	2020	Naturalised	H	8	9	5	6	5	6	0	6
Crataegus_monogyna	2020	Eurasiat	P	6	7	5	4	6	3	0	4
Crepis_bursifolia	2020	Others	H	9	6	4	3	8	2	0	7
Crepis_leontodontoides	2020	Others	H	5	8	4	4	3	7	0	3
Crepis_sancta_subsp_sancta	2020	Eurimedit	T	11	9	6	2	X	2	0	6
Crepis_vesicaria	2020	Eurimedit	T	8	8	3	3	6	2	0	6
Crithmum_maritimum	2020	Eurimedit	Ch	11	8	2	1	X	1	3	3
Cupressus_sempervirens	2020	Eurimedit	P	7	7	6	3	X	3	0	
Cuscuta_cesattiana	2020	Naturalised	T	8	7	5	X	X	X	0	6
Cuscuta_planiflora	2020	Eurimedit	T	8	7	5	X	X	X	0	
Cyclamen_hederifolium	2020	Stenomedit	G	4	8	5	5	5	5	0	1
Cyclamen_repandum_subsp_repandum	2020	Stenomedit	G	4	9	5	3	X	5	0	1

Cymbalaria_muralis	2020	Eurimedit	H	7	7	5	2	5	3	0	5
Cynodon_dactylon	2020	Cosmopol	G	8	8	5	4	X	4	0	7
Cynoglossum_creticum	2020	Eurimedit	H	11	9	5	3	X	7	0	6
Cynosurus_cristatus	2020	Eurasiat	H	8	5	4	5	5	4	0	6
Dactylis_glomerata_subsp_glomerata	2020	Eurasiat	H	7	6	5	4	5	6	0	5
Dactylis_hispanica_subsp_hispanica	2020	Stenomedit	H	11	8	4	2	5	2	0	3
Dasypyrum_villosum	2020	Eurimedit	T	8	10	5	2	4	2	0	6
Daucus_carota_subsp_carota	2020	Eurasiat	H	8	6	5	4	5	4	0	6
Dianthus_armeria_subsp_armeria	2020	Eurasiat	H	8	6	5	3	3	2	0	4
Digitaria_sanguinalis	2020	Cosmopol	T	7	7	5	3	6	4	0	7
Dioscorea_communis	2020	Eurimedit	G	5	7	5	5	8	6	0	1
Diplotaxis_erucoides_subsp_erucoides	2020	Stenomedit	T	8	8	4	3	5	5	0	6
Dipsacus_fullonum	2020	Eurimedit	H	6	8	5	7	5	5	0	6
Dittrichia_viscosa_subsp_viscosa	2020	Eurimedit	H	11	8	5	3	7	9	0	6
Ecballium_elaterium	2020	Eurimedit	G	7	8	5	3	5	3	0	6
Echium_italicum_subsp_italicum	2020	Eurimedit	H	11	8	5	3	3	4	0	6
Echium_plantagineum	2020	Eurimedit	T	11	8	5	3	5	5	0	6
Eleocharis_palustris_subsp_palustris	2020	Cosmopol	G	8	6	5	1	3	3	0	6
Elymus_repens_subsp_repens	2020	Circumbor	G	7	X	7	5	X	8	0	6
Epilobium_tetragonum	2020	Eurasiat	H	7	7	5	5	5	5	0	7
Equisetum_ramosissimum	2020	Circumbor	G	7	7	6	3	7	1	0	6
Erica_arborea	2020	Stenomedit	P	6	8	4	3	2	1	0	3
Erigeron_bonariensis	2020	Naturalised	T	8	8	5	3	X	7	0	7
Erigeron_sumatrensis	2020	Naturalised	T	8	8	5	3	X	7	0	7
Erodium_acaule	2020	Others	H	11	8	3	3	3	3	0	6
Erodium_cicutarium	2020	Cosmopol	H	8	7	5	3	5	3	0	6
Erodium_malacoides_subsp_malacoides	2020	Stenomedit	T	11	9	4	2	5	2	0	6
Erodium_moschatum	2020	Eurimedit	T	11	9	5	2	5	2	0	7
Ervilia_hirsuta	2020	Eurasiat	T	7	5	5	X	X	X	0	3
Ervum_gracile	2020	Eurimedit	T	7	8	5	4	4	4	0	5
Ervum_pubescens	2020	Eurimedit	T	8	8	5	3	4	2	0	6
Eryngium_campestre	2020	Eurimedit	H	9	7	5	3	8	3	0	4
Eryngium_maritimum	2020	Eurimedit	G	11	8	3	4	7	1	1	2
Euonymus_europaeus	2020	Eurasiat	P	6	5	5	5	8	5	0	2
Euphorbia_amygdaloides	2020	Eurasiat	Ch	4	5	4	5	7	6	0	2
Euphorbia_cuneifolia	2020	Stenomedit	T	7	7	4	6	7	4	0	5
Euphorbia_exigua_subsp_exigua	2020	Eurimedit	T	11	9	5	2	6	1	0	6
Euphorbia_helioscopia_subsp_helioscopia	2020	Cosmopol	T	9	7	5	3	5	6	0	6
Euphorbia_peplus	2020	Circumbor	T	6	7	4	4	5	7	0	6
Euphorbia_platyphyllos	2020	Eurimedit	T	6	7	5	5	5	6	0	6
Euphorbia_prostrata	2020	Naturalised	T	7	8	5	2	5	4	0	7
Festuca_danthonii_subsp_danthonii	2020	Eurimedit	T	8	9	5	2	4	2	0	6
Festuca_fasciculata	2020	Eurimedit	T	11	10	3	1	X	1	1	6
Festuca_geniculata	2020	Stenomedit	T	8	9	4	2	4	2	0	6
Festuca_ligustica	2020	Stenomedit	T	8	9	4	2	4	2	0	6
Festuca_myuros	2020	Cosmopol	T	8	9	5	2	6	2	0	6
Ficaria_verna_subsp_ficariiformis	2020	Eurimedit	G	4	5	5	6	7	7	0	3
Ficaria_verna_subsp_verna	2020	Eurasiat	G	4	5	5	6	7	7	0	3
Ficus_carica	2020	Eurimedit	P	7	8	6	X	5	X	0	3
Filago_germanica	2020	Eurasiat	T	8	7	5	3	4	2	0	5
Foeniculum_vulgare_subsp_piperitum	2020	Eurimedit	H	9	8	5	3	7	7	0	6
Fraxinus_angustifolia_subsp_oxycarpa	2020	Eurasiat	P	4	8	6	7	7	8	0	2
Fraxinus_ornus_subsp_ornus	2020	Eurasiat	P	5	8	6	3	8	3	0	3
Fumaria_capreolata_subsp_capreolata	2020	Eurimedit	T	7	9	5	3	5	3	0	5
Fumaria_officinalis	2020	Eurasiat	T	7	7	5	4	5	6	0	6
Galactites_tomentosus	2020	Stenomedit	H	8	8	4	3	X	7	0	6
Galium_aparine	2020	Eurasiat	T	6	X	5	4	5	5	0	4
Galium_parisiense	2020	Eurimedit	T	11	8	5	2	3	1	0	6
Gastridium_ventricosum	2020	Stenomedit	T	8	9	4	2	4	2	0	5
Gaudinia_fragilis	2020	Eurimedit	T	8	8	5	3	5	3	0	6
Geranium_columbinum	2020	Eurasiat	T	7	9	6	2	5	2	0	4
Geranium_dissectum	2020	Eurasiat	T	7	8	5	2	5	2	0	6
Geranium_molle	2020	Eurasiat	T	7	6	5	3	5	4	0	6
Geranium_purpureum	2020	Cosmopol	T	4	6	5	4	5	5	0	3
Geranium_rotundifolium	2020	Eurasiat	T	7	8	5	3	6	3	0	6
Geum_urbanum	2020	Circumbor	H	4	5	5	5	6	7	0	3

Gladiolus_italicus	2020	Eurimedit	G	9	9	5	3	5	3	0	4
Hedera_helix_subsp_helix	2020	Eurimedit	P	4	5	4	5	X	X	0	1
Heliotropium_europaeum	2020	Eurimedit	T	11	8	5	3	7	2	1	7
Helminthotheca_echioides	2020	Eurimedit	T	11	8	5	2	X	2	0	6
Holcus_lanatus_subsp_lanatus	2020	Circumbor	H	7	5	4	6	X	4	0	6
Hordeum_bulbosum	2020	Cosmopol	H	8	10	5	4	5	4	0	6
Hordeum_murinum_subsp_leporinum	2020	Eurimedit	T	9	9	5	3	5	3	0	7
Hymenocarpos_circinnatus	2020	Stenomedit	H	11	9	4	2	2	2	0	5
Hyoseris_radiata	2020	Stenomedit	H	11	8	4	2	7	1	0	3
Hypericum_australe	2020	Stenomedit	H	7	8	4	7	6	4	0	4
Hypericum_perforatum_subsp_veronense	2020	Eurasiat	H	7	8	6	X	X	X	0	5
Hypochoeris_achyrophorus	2020	Stenomedit	T	11	9	4	2	X	2	0	5
Hypochoeris_radicata	2020	Eurasiat	H	9	8	4	2	X	1	0	6
Isoetes_duriei	2020	Stenomedit	G	7	9	4	10	1	1	0	8
Isoetes_gymnocarpa	2020	Atlant	G								
Isoetes_histrix	2020	Stenomedit	G	7	10	4	10	1	1	0	7
Isoetes_sicula	2020		G								
Isolepis_cernua	2020	Cosmopol	T	8	6	5	9	4	1	0	7
Jacobaea_aquatica	2020	Eurasiat	H	7	6	5	8	4	5	0	
Jacobaea_erratica	2020	Eurasiat	H	7	6	4	4	7	4	0	6
Juncus_articulatus_subsp_articulatus	2020	Circumbor	G	8	7	4	8	6	5	0	6
Juncus_bufonius	2020	Cosmopol	T	4	7	5	6	4	1	0	7
Juncus_capitatus	2020	Eurimedit	T	8	10	2	8	4	1	1	7
Juncus_conglomeratus	2020	Circumbor	H	7	7	4	8	6	5	0	6
Juncus_effusus_subsp_effusus	2020	Cosmopol	H	7	7	5	9	6	5	0	6
Juncus_heterophyllus	2020	Atlant	I	7	7	3	10	4	3	0	3
Juncus_hybridus	2020	Eurimedit	T	8	8	3	8	6	3	0	7
Juncus_inflexus_subsp_inflexus	2020	Stenomedit	H	6	8	3	9	6	5	0	2
Juncus_subnodulosus	2020	Eurasiat	G	8	6	4	9	6	5	0	6
Kickxia_commutata_subsp_commutata	2020	Stenomedit	H	8	7	4	4	5	4	0	6
Kickxia_elatine_subsp_elatine	2020	Eurimedit	T	8	7	5	4	5	4	0	6
Knautia_integrifolia_subsp_integrifolia	2020	Eurimedit	T	7	8	5	3	3	2	0	6
Lactuca_sativa_subsp_serriola	2020	Eurasiat	H	9	7	7	4	6	4	0	7
Lagurus_ovatus_subsp_ovatus	2020	Eurimedit	T	8	9	5	3	X	2	1	5
Lamium_amplexicaule	2020	Eurasiat	T	7	7	5	4	5	7	0	7
Lamium_bifidum	2020	Stenomedit	T	7	8	4	3	4	3	0	3
Lamium_purpureum	2020	Eurasiat	T	7	7	5	4	5	5	0	7
Lathyrus_annuus	2020	Eurimedit	T	8	8	5	3	5	2	0	6
Lathyrus_aphaca_subsp_aphaca	2020	Eurimedit	T	6	6	5	3	X	X	0	3
Lathyrus_clymenum	2020	Stenomedit	T	7	8	4	4	3	3	0	4
Lathyrus_ochrus	2020	Stenomedit	T	7	8	4	2	5	2	0	6
Lathyrus_oleraceus	2020	Atlant	T	9	9	4	3	4	3	0	6
Lathyrus_sphaericus	2020	Eurimedit	T	11	9	5	2	5	2	0	4
Laurus_nobilis	2020	Stenomedit	P	2	7	4	8	4	6	0	4
Lemna_minuta	2020	Naturalised	I	7	6	5	12	7	8	0	7
Ligustrum_vulgare	2020	Eurasiat	NP	7	6	4	X	8	X	0	3
Limbarda_crithmoides	2020	Stenomedit	Ch	11	8	4	7	9	5	3	5
Limonium_narbonense	2020	Eurimedit	H	11	7	5	6	7	5	3	6
Linaria_vulgaris_subsp_vulgaris	2020	Eurasiat	H	8	5	5	3	7	3	0	6
Linum_trigynum	2020	Eurimedit	T	11	9	5	2	3	2	0	5
Linum_usitatissimum_subsp_angustifolium	2020	Eurimedit	H	7	7	5	3	7	2	0	6
Lolium_arundinaceum_subsp_arundinaceum	2020	Eurimedit	H	9	8	5	6	8	6	0	6
Lolium_multiflorum	2020	Eurimedit	T	7	7	5	4	X	6	0	6
Lolium_perenne	2020	Circumbor	H	8	5	4	5	X	7	0	6
Lolium_rigidum_subsp_rigidum	2020	Cosmopol	T	8	8	5	3	4	2	0	6
Loncomelos_narbonense	2020	Eurimedit	G	8	7	5	4	6	4	0	5
Lonicera_caprifolium	2020	Eurasiat	P	6	5	6	6	X	5	0	1
Lonicera_japonica	2020	Naturalised	P	6	5	6	6	X	5	0	3
Lotus_angustissimus	2020	Eurimedit	T	11	8	5	7	7	4	0	6
Lotus_ornithopodioides	2020	Stenomedit	T	11	9	4	2	1	1	0	5
Lotus_tenuis	2020	Eurasiat	H	9	7	5	6	7	7	0	6
Lotus_tetragonolobus	2020	Stenomedit	T	8	6	4	6	9	6	0	4
Lupinus_gussoneanus	2020	Stenomedit	T	11	9	4	2	2	2	0	3
Luzula_forsteri	2020	Eurimedit	H	4	7	5	4	4	5	0	2
Lychnis_flos-cuculi	2020	Circumbor	H	7	5	4	6	X	6	0	3
Lysimachia_arvensis	2020	Eurimedit	T	6	6	5	5	X	6	0	6

Lysimachia_foemina	2020	Cosmopol	T	8	7	5	4	9	5	0	5
Lysimachia_nardii	2020	Stenomedit	T	7	8	4	5	2	1	0	6
Lysimachia_nummularia	2020	Eurasiat	H	4	6	4	6	X	X	0	
Lythrum_hyssopifolia	2020	Cosmopol	T	8	7	5	7	3	4	0	7
Lythrum_junceum	2020	Stenomedit	H	7	8	4	7	3	4	0	7
Lythrum_salicaria	2020	Cosmopol	H	7	5	5	8	7	X	0	7
Lythrum_tribracteatum	2020	Eurimedit	T	7	8	5	7	3	4	0	5
Malope_malacoides	2020	Stenomedit	T	9	9	5	2	5	4	0	6
Malus_sylvestris	2020	Eurasiat	P	7	5	5	5	7	5	0	3
Malva_multiflora	2020	Stenomedit	T	8	9	4	2	5	4	0	7
Malva_punctata	2020	Stenomedit	T	8	9	4	2	5	4	0	6
Malva_sylvestris	2020	Circumbor	H	8	6	4	4	X	8	0	7
Matthiola_sinuata	2020	Stenomedit	H	11	10	4	2	X	1	2	3
Medicago_arabica	2020	Eurimedit	T	9	9	5	2	X	2	0	7
Medicago_doliata	2020	Stenomedit	T	11	9	4	2	X	2	0	6
Medicago_lupulina	2020	Eurasiat	T	7	5	X	4	8	7	0	6
Medicago_minima	2020	Eurimedit	T	11	7	5	3	8	1	0	5
Medicago_murex	2020	Stenomedit	T	11	9	4	2	X	2	0	6
Medicago_polymorpha	2020	Eurimedit	T	9	9	5	2	X	2	0	6
Medicago_praecox	2020	Stenomedit	T	11	9	4	2	X	2	0	6
Medicago_rigidula	2020	Eurimedit	T	11	8	5	1	X	1	2	5
Medicago_truncatula	2020	Stenomedit	T	11	8	4	1	X	1	2	5
Melica_minuta_subsp_latifolia	2020	Stenomedit	H	8	10	4	2	5	2	0	2
Melica_uniflora	2020	Eurasiat	H	3	5	5	5	6	X	0	1
Melissa_officinalis_subsp_altissima	2020	Stenomedit	H	6	7	5	4	6	4	0	6
Mentha_aquatica_subsp_aquatica	2020	Eurasiat	H	7	5	5	9	7	4	0	7
Mentha_pulegium_subsp_pulegium	2020	Eurimedit	H	8	7	5	7	X	2	0	6
Mercurialis_annua	2020	Eurasiat	T	7	7	5	4	7	8	0	6
Muscari_comosum	2020	Eurimedit	G	7	8	5	3	7	0	0	4
Myosotis_ramosissima_subsp_ramosissima	2020	Eurasiat	T	9	8	5	2	4	3	0	6
Myrtus_communis	2020	Stenomedit	P	8	9	4	3	5	2	0	2
Narcissus_tazetta_subsp_tazetta	2020	Stenomedit	G	8	8	4	4	5	4	0	7
Oenanthe_pimpinelloides	2020	Eurimedit	H	5	7	3	4	5	4	0	3
Oloptum_miliaceum	2020	Stenomedit	H	5	7	4	4	7	5	0	5
Ophrys_apifera	2020	Eurimedit	G	7	6	5	4	9	2	0	4
Ophrys_bombyliflora	2020	Stenomedit	G	8	9	4	3	6	3	0	
Ophrys_sphegodes_subsp_sphegodes	2020	Eurimedit	G	8	8	5	4	9	3	0	4
Opuntia_ficus-indica	2020	Naturalised	P	9	8	6	2	X	2	0	1
Ornithopus_compressus	2020	Eurimedit	T	11	9	5	2	2	1	0	6
Orobanche_artemisiae-campestris	2020	Eurimedit	T	7	8	5	3	6	4	0	6
Orobanche_crenata	2020	Eurimedit	T	8	5	6	3	5	4	0	4
Orobanche_hederae	2020	Eurimedit	T	6	7	5	4	5	5	0	1
Orobanche_minor	2020	Eurasiat	T	7	6	5	4	5	4	0	6
Oxalis_articulata	2020	Naturalised	G	8	9	4	3	4	5	0	6
Oxalis_corniculata	2020	Eurimedit	H	7	7	0	4	X	6	0	7
Oxalis_pes-caprae	2020	Naturalised	G	8	10	4	3	X	5	0	6
Paliurus_spina-christi	2020	Eurasiat	P	7	8	6	3	7	3	0	4
Pancratium_maritimum	2020	Stenomedit	G	11	10	3	1	X	1	0	2
Papaver_rhoeas_subsp_rhoeas	2020	Others	T	6	6	5	5	7	X	0	6
Parapholis_pycnantha	2020	Atlant	T	10	8	6	4	8	3	5	4
Parietaria_judaica	2020	Eurimedit	H	7	8	5	3	X	6	0	5
Paspalum_dilatatum	2020	Naturalised	H	X	8	X	10	8	8	0	6
Paspalum_distichum	2020	Naturalised	G	X	8	X	10	8	8	0	7
Passiflora_caerulea	2020	Naturalised	P	6	6	5	5	5	5	0	9
Petrorhagia_dubia	2020	Stenomedit	T	11	8	5	2	8	2	0	6
Phalaris_aquatica	2020	Stenomedit	H	7	7	X	4	6	4	0	6
Phalaris_coerulescens	2020	Stenomedit	H	7	6	X	5	6	6	0	6
Phalaris_truncata	2020	Eurimedit	H	7	7	X	4	6	4	0	6
Phillyrea_angustifolia	2020	Stenomedit	P	11	10	4	1	X	2	0	2
Phillyrea_latifolia	2020	Stenomedit	P	5	8	4	4	X	5	0	4
Phleum_pratense_subsp_pratense	2020	Circumbor	H	7	6	5	5	6	6	0	6
Phoenix_canariensis	2020	Naturalised	P	11	10	2	4	X	4	0	9
Phragmites_australis	2020	Cosmopol	He	7	5	X	10	7	5	1	6
Picris_hieracioides	2020	Circumbor	H	8	X	5	4	8	4	0	6
Pinus_halepensis	2020	Stenomedit	P	11	10	4	2	0	2	0	1
Pinus_pinea	2020	Eurimedit	P	11	8	5	2	4	3	0	2

Pistacia_lentiscus	2020	Stenomedit	P	11	10	5	2	X	2	0	2
Pittosporum_tobira	2020	Naturalised	P	10	9	5	2	X	2	0	
Plantago_coronopus	2020	Eurimedit	T	8	7	5	7	7	4	0	6
Plantago_lanceolata	2020	Eurasiat	H	6	7	5	X	X	X	0	6
Plantago_macrorhiza	2020	Stenomedit	H	11	10	4	3	9	2	1	4
Plantago_major	2020	Eurasiat	H	8	X	X	5	X	7	0	7
Poa_annua	2020	Cosmopol	T	7	X	5	6	X	8	0	8
Poa_bulbosa	2020	Eurasiat	H	8	8	7	2	4	1	0	6
Poa_trivialis	2020	Eurasiat	H	6	X	5	7	X	7	0	6
Polycarpon_tetraphyllum_subsp_tetraphyllum	2020	Eurimedit	T	7	7	5	4	5	6	0	8
Polygala_monspeliaca	2020	Stenomedit	T	8	8	4	5	7	1	0	4
Polygonum_aviculare_subsp_aviculare	2020	Cosmopol	T	7	7	5	3	6	1	0	7
Polygonum_maritimum	2020	Cosmopol	Ch	11	10	4	1	3	1	2	5
Polygonum_romanum	2020	Others	Ch	11	10	4	2	2	2	0	7
Polypogon_monspeliensis	2020	Cosmopol	T	8	8	5	9	8	6	0	6
Portulaca_oleracea	2020	Cosmopol	T	7	8	5	4	7	7	0	7
Potamogeton_nodosus	2020	Cosmopol	I	6	6	5	12	7	6	0	9
Potentilla_reptans	2020	Eurasiat	H	6	6	5	6	7	5	0	6
Poterium_sanguisorba_subsp_balearicum	2020	Eurasiat	H	7	6	5	3	8	2	0	4
Prospero_autumnale	2020	Eurimedit	G	8	8	4	2	6	3	0	5
Prunella_laciniata	2020	Eurimedit	H	8	8	5	3	7	2	0	4
Prunella_vulgaris_subsp_vulgaris	2020	Circumbor	H	7	6	4	6	4	X	0	3
Prunus_spinosa_subsp_spinosa	2020	Eurasiat	P	7	5	5	X	X	X	0	4
Pteridium_aquilinum_subsp_aquilinum	2020	Cosmopol	G	6	5	4	6	3	3	0	3
Pulicaria_dysenterica	2020	Eurimedit	H	8	6	5	7	X	5	0	7
Pulicaria_odora	2020	Eurimedit	H	5	8	5	4	X	4	0	3
Pulicaria_vulgaris	2020	Eurasiat	T	7	7	5	7	7	7	0	7
Pyrus_spinosa	2020	Stenomedit	P	7	8	4	4	7	3	0	4
Quercus_cerris	2020	Eurimedit	P	6	8	5	4	4	4	0	2
Quercus_frainetto	2020	Eurasiat	P	7	6	6	6	5	6	0	1
Quercus_ilex	2020	Stenomedit	P	2	9	4	3	X	X	0	2
Quercus_petraea	2020	Eurasiat	P	6	6	5	5	4	6	0	3
Quercus_pubescens_subsp_pubescens	2020	Eurasiat	P	7	8	6	3	7	4	0	2
Quercus_robur	2020	Eurasiat	P	7	6	6	6	5	6	0	2
Quercus_suber	2020	Eurimedit	P	4	8	3	3	3	3	0	1
Quercus_virgiliana	2020	Eurasiat	P	7	8	6	4	7	5	0	
Ranunculus_bulbosus	2020	Eurasiat	H	8	6	5	3	7	3	0	6
Ranunculus_ophioglossifolius	2020	Eurimedit	T	7	7	5	8	6	6	0	7
Ranunculus_sardous	2020	Eurimedit	T	8	7	5	8	X	7	0	7
Ranunculus_velutinus	2020	Eurimedit	H	6	8	5	5	6	5	0	6
Raphanus_raphanistrum_subsp_raphanistrum	2020	Eurimedit	T	11	5	5	X	4	5	0	6
Reichardia_picroides	2020	Stenomedit	H	7	8	4	3	6	2	0	4
Rhagadiolus_edulis	2020	Eurimedit	T	7	8	5	4	5	4	0	4
Rhamnus_alaternus_subsp_alaternus	2020	Eurimedit	P	4	9	5	2	4	4	0	3
Robinia_pseudoacacia	2020	Naturalised	P	5	7	5	4	X	8	0	6
Romulea_bulbocodium	2020	Stenomedit	G	8	9	4	3	4	3	0	5
Romulea_rollii	2020	Stenomedit	G	9	9	3	3	5	2	0	4
Rosa_sempervirens	2020	Stenomedit	NP	6	8	4	3	4	6	0	2
Rostraria_pubescens	2020	Stenomedit	T	7	8	4	5	8	2	0	4
Rubia_peregrina	2020	Stenomedit	P	5	9	4	4	5	3	0	1
Rubus_caesius	2020	Eurasiat	NP	7	5	5	7	7	9	0	3
Rubus_ulmifolius	2020	Eurimedit	NP	5	8	5	4	5	8	0	3
Rumex_acetosa_subsp_acetosa	2020	Circumbor	H	8	X	X	X	4	5	0	3
Rumex_acetosella_subsp_pyrenaicus	2020	Cosmopol	H	8	5	5	5	1	2	0	6
Rumex_conglomeratus	2020	Eurasiat	H	8	7	5	7	X	8	0	6
Rumex_crispus	2020	Cosmopol	H	7	5	5	6	X	5	0	7
Rumex_sanguineus	2020	Eurasiat	H	4	5	4	8	7	7	0	4
Ruscus_aculeatus	2020	Eurimedit	G	4	8	5	4	5	5	0	1
Salsola_tragus	2020	Eurasiat	T	9	7	8	8	7	8	2	3
Salvia_verbenaca	2020	Stenomedit	H	8	8	4	3	5	7	0	6
Sambucus_nigra	2020	Eurasiat	P	7	5	4	5	X	9	0	5
Schoenoplectus_lacustris	2020	Cosmopol	G	8	5	5	11	7	5	0	
Scirpoides_holoschoenus	2020	Eurimedit	G	8	8	5	8	5	4	0	4
Scolymus_hispanicus	2020	Eurimedit	H	11	8	5	3	X	2	0	6
Scorpiurus_muricatus	2020	Eurimedit	T	7	8	5	2	X	2	0	4
Sedum_cepaea	2020	Eurimedit	T	2	8	2	4	2	4	0	3

Senecio_vulgaris	2020	Eurimedit	T	7	X	X	5	X	8	0	7
Serapias_lingua	2020	Stenomedit	G	11	8	4	3	4	2	0	6
Serapias_parviflora	2020	Stenomedit	G	11	10	4	2	4	2	0	5
Serapias_vomeracea	2020	Eurimedit	G	11	8	5	3	4	2	0	4
Setaria_verticillata	2020	Cosmopol	T	7	8	5	4	X	8	0	7
Sherardia_arvensis	2020	Eurimedit	T	8	6	5	5	8	5	0	6
Silene_bellidifolia	2020	Stenomedit	T	7	8	5	2	2	1	0	6
Silene_canescens	2020	Stenomedit	T	11	9	3	1	X	1	2	5
Silene_gallica	2020	Eurimedit	T	8	9	5	3	2	1	0	6
Silene_latifolia	2020	Stenomedit	H	6	9	4	3	4	2	0	3
Silene_vulgaris_subsp_tenoreana	2020	Eurasiat	H	8	X	X	4	7	2	0	5
Silybum_marianum	2020	Eurimedit	H	11	10	6	3	5	7	0	6
Sinapis_arvensis_subsp_arvensis	2020	Stenomedit	T	7	5	4	X	8	6	0	6
Sisymbrium_officinale	2020	Eurasiat	T	8	6	5	4	X	7	0	7
Sixalix_atropurpurea	2020	Stenomedit	H	6	8	4	3	X	2	0	5
Smilax_aspera	2020	Cosmopol	NP	6	10	4	2	5	3	0	1
Solanum_nigrum	2020	Cosmopol	T	7	6	5	3	5	7	0	7
Solenopsis_laurentia	2020	Stenomedit	T	7	8	4	7	2	1	0	7
Sonchus_asper_subsp_asper	2020	Eurasiat	T	7	5	X	4	7	7	0	6
Sonchus_bulbosus_subsp_bulbosus	2020	Stenomedit	G	7	8	4	3	5	3	0	3
Sonchus_oleraceus	2020	Eurasiat	T	7	5	X	4	8	8	0	6
Sonchus_tenerrimus	2020	Stenomedit	T	7	8	4	2	5	4	0	6
Sorbus_domestica	2020	Eurimedit	P	4	7	5	3	8	3	0	1
Sorbus_torminalis	2020	Eurasiat	P	4	6	5	4	7	4	0	1
Sorghum_halepense	2020	Cosmopol	G	8	8	X	7	8	8	0	6
Spartium_junceum	2020	Eurimedit	P	7	7	5	4	7	2	0	4
Spergularia_media	2020	Cosmopol	T	7	7	5	7	8	5	3	5
Spiranthes_spiralis	2020	Eurasiat	G	8	6	4	3	X	3	0	
Sporobolus_virginicus	2020	Cosmopol	G	11	11	4	1	0	1	3	3
Stachys_ocymastrum	2020	Stenomedit	T	11	9	4	2	7	2	0	6
Stachys_sylvatica	2020	Circumbor	H	5	X	4	7	7	7	0	3
Stellaria_media	2020	Cosmopol	T	7	X	X	4	7	8	0	6
Stellaria_neglecta	2020	Eurasiat	T	6	7	5	4	5	8	0	4
Stellaria_pallida	2020	Eurasiat	T	8	8	5	3	5	4	0	7
Symphyotrichum_squamatum	2020	Naturalised	T	8	8	5	4	7	7	0	7
Symphytum_bulbosum	2020	Eurasiat	G	4	7	6	4	5	3	0	2
Tamarix_canariensis	2020	Stenomedit	P	11	9	4	6	5	3	1	
Taraxacum_officinalis	2020	Circumbor	H	7	X	X	5	X	7	0	7
Thinopyrum_acutum	2020	Eurimedit	G	11	7	5	5	7	7	2	6
Thinopyrum_junceum	2020	Eurimedit	G	11	6	5	7	7	7	2	2
Thymelaea_passerina	2020	Eurimedit	T	8	7	5	3	7	2	0	5
Tordylium_apulum	2020	Stenomedit	T	11	9	4	2	X	3	0	6
Torilis_arvensis	2020	Cosmopol	T	7	8	5	4	7	6	0	4
Torilis_nodosa	2020	Eurimedit	T	7	8	6	4	7	6	0	6
Trifolium_angustifolium_subsp_angustifolium	2020	Eurimedit	T	11	9	5	2	3	2	0	5
Trifolium_arvense	2020	Eurasiat	T	8	5	5	2	2	1	0	6
Trifolium_campestre	2020	Eurasiat	T	8	5	5	4	X	4	0	6
Trifolium_echinatum	2020	Eurasiat	T	8	9	6	2	2	1	0	6
Trifolium_fragiferum_subsp_fragiferum	2020	Eurasiat	H	8	6	5	7	8	7	0	6
Trifolium_incarnatum_subsp_incarnatum	2020	Eurimedit	T	11	8	5	4	5	7	0	6
Trifolium_lappaceum	2020	Eurimedit	T	8	9	5	2	2	1	0	5
Trifolium_ligusticum	2020	Stenomedit	T	8	9	4	2	2	1	0	6
Trifolium_nigrescens_subsp_nigrescens	2020	Eurimedit	T	8	6	5	5	5	6	0	6
Trifolium_pallidum	2020	Eurimedit	T	7	8	5	4	2	2	0	6
Trifolium_pratense_subsp_pratense	2020	Circumbor	H	7	X	4	X	X	X	0	6
Trifolium_repens	2020	Eurasiat	H	8	X	X	X	X	7	0	7
Trifolium_resupinatum	2020	Eurasiat	T	8	8	5	5	X	5	0	6
Trifolium_scabrum	2020	Eurimedit	T	11	8	5	2	9	1	0	5
Trifolium_sebastiani	2020	Stenomedit	T	8	9	6	3	2	2	0	7
Trifolium_squamosum	2020	Eurimedit	T	11	8	5	6	7	6	0	5
Trifolium_squarrosum	2020	Eurimedit	T	11	9	5	2	3	2	0	6
Trifolium_stellatum	2020	Eurimedit	T	11	9	5	2	X	2	0	4
Trifolium_subterraneum	2020	Eurimedit	T	11	9	5	2	2	2	0	6
Trifolium_vesiculosum	2020	Eurimedit	T	8	9	5	3	5	2	0	6
Trigonella_alba	2020	Eurasiat	T	9	6	6	3	7	3	0	6
Trigonella_smalii	2020	Eurimedit	H	7	7	4	4	5	5	0	6

Triticum_vagans	2020	Stenomedit	T	11	10	X	5	5	4	0	4
Typha_angustifolia	2020	Circumbor	G	8	7	5	10	X	7	0	6
Typha_latifolia	2020	Cosmopol	G	8	6	5	10	X	8	0	4
Tyrimnus_leucographus	2020	Stenomedit	T	7	9	4	3	5	7	0	4
Ulmus_minor	2020	Eurasiat	P	5	7	5	X	8	X	0	4
Urospermum_dalechampii	2020	Eurimedit	H	8	8	5	3	X	3	0	4
Urtica_membranacea	2020	Stenomedit	T	7	8	5	3	6	3	0	6
Valerianella_eriocarpa	2020	Stenomedit	T	11	9	4	2	5	1	0	6
Verbascum_blattaria	2020	Eurasiat	H	8	6	7	3	7	6	0	6
Verbascum_blattaria_x_sinuatum	2020		H								
Verbascum_sinuatum	2020	Eurimedit	H	9	8	5	3	7	7	0	6
Verbena_officinalis	2020	Eurasiat	H	9	5	5	4	X	6	0	6
Veronica_arvensis	2020	Eurasiat	T	5	5	5	5	6	X	0	7
Veronica_cymbalaria	2020	Eurimedit	T	7	7	5	4	3	2	0	3
Veronica_hederifolia	2020	Eurasiat	T	6	6	5	5	3	7	0	6
Veronica_persica	2020	Naturalised	T	8	7	5	5	5	6	0	7
Veronica_serpyllifolia	2020	Eurasiat	H	X	X	5	3	5	X	0	3
Viburnum_tinus_subsp_tinus	2020	Stenomedit	P	5	9	4	4	5	3	0	2
Vicia_angustifolia	2020	Eurimedit	T	5	5	6	X	X	X	0	6
Vicia_benghalensis	2020	Stenomedit	T	11	9	4	2	5	2	0	7
Vicia_bithynica	2020	Eurimedit	T	7	7	5	3	5	5	0	5
Vicia_disperma	2020	Stenomedit	T	11	10	4	2	2	1	0	4
Vicia_grandiflora	2020	Eurasiat	H	7	8	6	3	5	4	0	2
Vicia_hybrida	2020	Eurimedit	T	7	8	5	3	5	5	0	6
Vicia_lutea	2020	Eurimedit	T	7	8	5	3	5	5	0	6
Vicia_narbonensis	2020	Eurimedit	T	7	8	5	3	5	5	0	7
Vicia_segetalis	2020	Eurimedit	T	5	5	6	X	X	X	0	6
Vinca_major_subsp_major	2020	Eurimedit	Ch	6	7	5	4	5	3	0	3
Viola_alba_subsp_deinhardtii	2020	Eurimedit	H	5	8	5	5	7	6	0	1
Viola_reichenbachiana	2020	Circumbor	H	4	5	4	5	7	6	0	2
Viola_suavis	2020	Eurasiat	H	5	8	6	5	4	4	0	2
Vitis_vinifera	2020	Cosmopol	P	6	8	5	6	8	6	0	2
Xanthium_italicum	2020	Eurimedit	T	8	8	5	5	X	1	0	6

DATASET: "Lucchese 1990"

Species	**Year**	**Chorotype**	**Main life form**	**L**	**T**	**K**	**F**	**R**	**N**	**S**	**H**
Acer_campestre	1990	Eurasiat	P	5	7	4	5	7	6	0	3
Acer_monspessulanum	1990	Eurimedit	P	6	8	5	3	8	4	0	1
Agrimonia_eupatoria_subsp_eupatoria	1990	Cosmopol	H	7	6	5	4	8	4	0	5
Agrostis_stolonifera	1990	Circumbor	H	8	X	X	6	X	5	0	6
Ailanthus_altissima	1990	Naturalised	P	6	7	5	5	5	5	0	6
Aira_cupaniana	1990	Stenomedit	T	8	9	4	2	3	1	0	6
Aira_elegantissima_subsp_elegantissima	1990	Eurimedit	T	8	9	5	2	3	1	0	6
Ajuga_iva	1990	Stenomedit	Ch	8	8	4	3	7	2	0	4
Alisma_plantagoaquatica	1990	Cosmopol	I	7	X	X	1	X	8	0	6
Allium_ampeloprasum	1990	Eurimedit	G	7	7	5	3	6	5	0	5
Allium_chamaemoly	1990	Stenomedit	G	8	10	4	2	4	2	0	5
Allium_roseum_subsp_roseum	1990	Stenomedit	G	8	8	4	3	6	5	0	5
Allium_triquetrum	1990	Stenomedit	G	6	9	4	4	4	7	0	1
Alopecurus_myosuroides_subsp_myosuroides	1990	Cosmopol	T	6	6	5	6	7	7	0	6
Alopecurus_rendlei	1990	Eurimedit	T	8	7	5	8	7	8	0	7
Althaea_cannabina	1990	Eurasiat	H	9	8	6	7	7	6	0	6
Althaea_officinalis	1990	Eurasiat	H	7	6	6	7	7	6	0	7
Amaranthus_blitoides	1990	Naturalised	T	9	7	7	3	X	9	0	7
Amaranthus_deflexus	1990	Naturalised	T	8	8	5	4	6	9	0	7
Amaranthus_hybridus_subsp_cruentus	1990	Cosmopol	T	8	8	5	4	6	8	0	7
Amaranthus_retroflexus	1990	Naturalised	T	9	9	7	4	X	9	0	7
Ammoides_pusilla	1990	Stenomedit	T	7	9	4	2	5	2	0	5
Anacamptis_laxiflora	1990	Eurimedit	G	8	7	5	6	6	5	0	6
Anacamptis_morio	1990	Eurasiat	G	7	5	4	4	7	3	0	5
Anacamptis_papilionacea	1990	Eurimedit	G	8	8	5	3	6	4	0	6
Anacamptis_pyramidalis	1990	Eurimedit	G	8	7	5	3	9	2	0	4
Anacyclus_radiatus_subsp_radiatus	1990	Stenomedit	T	8	9	4	4	5	2	0	7
Anemone_hortensis_subsp_hortensis	1990	Eurimedit	G	8	8	5	4	4	3	0	3
Anisantha_diandra	1990	Eurimedit	T	8	8	5	3	5	4	0	6

Anisantha_madritensis	1990	Eurimedit	T	8	7	5	3	X	1	0	6
Anisantha_rigida	1990	Cosmopol	T	8	8	5	4	6	5	0	6
Anisantha_rubens	1990	Stenomedit	T	8	11	5	2	X	2	0	6
Anthemis_arvensis_subsp_arvensis	1990	Stenomedit	T	7	6	4	4	3	6	0	6
Anthemis_maritima_subsp_maritima	1990	Others	H	11	9	4	1	X	1	0	3
Anthoxanthum_odoratum	1990	Eurasiat	H	X	X	5	X	5	3	0	4
Apium_graveolens	1990	Eurasiat	H	7	7	5	7	5	7	0	7
Arabis_sagittata	1990	Eurasiat	H	7	6	6	4	8	3	0	3
Arbutus_unedo	1990	Stenomedit	P	11	9	4	3	4	2	0	2
Arenaria_leptoclados_subsp_leptoclados	1990	Eurasiat	T	9	9	5	2	3	1	0	6
Arisarum_vulgare_subsp_vulgare	1990	Stenomedit	G	6	8	4	4	4	4	0	3
Arum_italicum_subsp_italicum	1990	Stenomedit	G	6	8	4	4	5	5	0	3
Arundo_plinii	1990	Stenomedit	G	11	8	4	4	4	2	0	4
Asparagus_acutifolius	1990	Stenomedit	G	6	9	4	2	5	5	0	2
Asparagus_officinalis_subsp_officinalis	1990	Eurimedit	G	8	8	5	5	5	5	0	7
Asphodelus_ramosus_subsp_ramosus	1990	Stenomedit	G	11	9	4	2	3	5	0	4
Asplenium_ceterach_subsp_bivalens	1990	Eurasiat	H	9	7	5	2	7	3	0	2
Asplenium_onopteris_	1990	Cosmopol	H	3	9	4	3	5	3	0	1
Asplenium_trichomanes_subsp_quadrivalens	1990	Cosmopol	H	5	X	5	5	X	4	0	2
Atriplex_halimus	1990	Stenomedit	P	11	10	4	1	6	2	3	4
Atriplex_patula	1990	Circumbor	T	6	5	X	5	7	X	0	7
Atriplex_patula_var_angustifolia	1990	Circumbor	T	6	5	X	5	7	X	0	7
Atriplex_prostrata	1990	Circumbor	T	9	X	X	6	X	9	0	7
Atriplex_rosea	1990	Eurimedit	T	9	9	7	2	6	1	1	
Avena_barbata_	1990	Eurimedit	T	8	8	5	3	7	2	0	6
Ballota_nigra_subsp_meridionalis	1990	Eurimedit	H	8	6	5	5	X	8	0	7
Barbarea_vulgaris	1990	Cosmopol	H	8	X	5	7	X	6	0	6
Bellardia_viscosa	1990	Eurimedit	T	8	8	3	3	3	3	0	6
Bellevalia_romana	1990	Eurimedit	G	8	7	5	3	6	4	0	6
Bellis_perennis	1990	Eurasiat	H	9	5	4	X	X	5	0	7
Bellis_sylvestris	1990	Stenomedit	H	5	8	4	3	3	3	0	3
Beta_vulgaris_subsp_vulgaris	1990	Eurimedit	H	11	7	5	6	6	5	1	6
Betonica_officinalis	1990	Eurasiat	H	6	5	4	6	4	3	0	2
Blackstonia_perfoliata_subsp_perfoliata	1990	Eurimedit	T	8	7	5	X	9	4	0	4
Bolboschoenus_maritimus	1990	Cosmopol	G	8	X	4	1	8	5	2	5
Borago_officinalis	1990	Eurimedit	T	7	8	5	3	5	5	0	6
Bothriochloa_ischaemum	1990	Cosmopol	H	9	7	5	3	8	3	0	5
Brachypodium_phoenicoides	1990	Stenomedit	G	8	8	4	3	8	4	0	4
Brachypodium_sylvaticum_subsp_sylvaticum	1990	Eurasiat	H	4	5	5	5	6	6	0	2
Briza_maxima	1990	Cosmopol	T	8	10	5	2	4	1	0	5
Briza_minor	1990	Cosmopol	T	8	9	5	2	4	1	0	6
Bromus_hordeaceus_subsp_hordeaceus	1990	Cosmopol	T	7	6	5	X	X	X	0	6
Bupleurum_tenuissimum	1990	Eurimedit	T	11	8	5	4	7	2	1	4
Cakile_maritima_subsp_maritima	1990	Eurimedit	T	9	8	2	6	X	8	2	3
Calamagrostis_arenaria_subsp_arundinacea	1990	Eurimedit	G	12	6	5	4	7	5	2	2
Calendula_arvensis	1990	Eurimedit	T	7	8	5	3	8	5	0	6
Campanula_erinus	1990	Stenomedit	T	7	8	4	2	X	1	0	4
Campanula_rapunculus	1990	Eurasiat	H	7	7	5	4	6	4	0	6
Capsella_rubella	1990	Eurimedit	T	8	9	5	2	4	2	0	7
Cardamine_hirsuta	1990	Cosmopol	T	7	8	5	3	5	4	0	6
Carduus_nutans_subsp_nutans	1990	Atlant	H	8	X	5	3	8	6	0	6
Carduus_pycnocephalus_subsp_pycnocephalus	1990	Eurimedit	H	7	8	4	3	X	3	0	6
Carex_distachya	1990	Stenomedit	H	6	6	4	2	4	5	0	1
Carex_divisa	1990	Eurimedit	G	8	8	2	3	5	3	0	6
Carex_divulsa_	1990	Eurimedit	H	7	6	5	4	5	5	0	5
Carex_flacca_subsp_flacca	1990	Eurasiat	G	7	5	5	6	8	X	0	3
Carex_grioletii	1990	Stenomedit	G	4	5	6	3	6	5	0	0
Carex_hallerana	1990	Eurimedit	H	5	7	5	3	3	4	0	1
Carex_otrubae	1990	Eurimedit	H	9	5	5	9	X	5	0	7
Carex_spicata	1990	Eurasiat	H	7	6	5	4	5	5	0	7
Carex_sylvatica	1990	Eurasiat	H	2	5	3	5	7	5	0	2
Carlina_corymbosa	1990	Stenomedit	H	6	X	4	2	X	4	0	5
Carpinus_betulus	1990	Eurasiat	P	4	6	4	X	X	X	0	1
Carthamus_caeruleus	1990	Eurimedit	H	11	11	5	3	7	4	0	5
Catapodium_balearicum	1990	Eurimedit	T	11	10	3	1	X	1	2	4
Catapodium_rigidum	1990	Eurimedit	T	8	8	5	2	5	4	0	6

Centaurea_jacea_subsp_gaudinii	1990	Eurasiat	H	6	5	7	4	7	3	0	6
Centaurea_pullata_subsp_pullata	1990	Stenomedit	T	9	8	4	3	8	6	3	4
Centaurea_sphaerocephala_subsp_sphaerocephala	1990	Stenomedit	H	11	10	4	1	X	1	0	6
Centaurium_erythraea_subsp_erythraea	1990	Eurasiat	H	8	6	5	5	6	X	0	4
Cephalanthera_longifolia	1990	Eurasiat	G	4	5	5	3	8	3	0	1
Cerastium_glomeratum	1990	Eurimedit	T	7	X	5	5	5	5	0	7
Cerastium_ligusticum	1990	Stenomedit	T	11	9	4	2	3	1	0	6
Cerinthe_major_subsp_major	1990	Stenomedit	T	7	8	4	4	5	9	0	6
Chamaeiris_foetidissima	1990	Eurimedit	G	7	7	5	4	4	5	0	2
Chamaerops_humilis	1990	Stenomedit	NP	11	10	3	1	4	1	0	1
Chenopodium_album	1990	Cosmopol	T	7	7	5	4	5	7	0	7
Chenopodium_vulvaria	1990	Eurimedit	T	7	7	5	4	X	9	0	7
Chondrilla_juncea	1990	Eurasiat	H	8	7	5	3	8	X	0	6
Cichorium_intybus	1990	Eurasiat	H	9	6	5	3	8	5	0	6
Cirsium_vulgare_subsp_vulgare	1990	Eurasiat	H	8	5	5	5	X	8	0	6
Clematis_flammula	1990	Eurimedit	P	7	9	5	3	5	4	0	2
Clematis_vitalba	1990	Eurasiat	P	7	7	4	5	7	7	0	3
Clinopodium_menthifolium_subsp_ascendens	1990	Eurasiat	H	4	6	4	5	5	4	0	2
Clinopodium_nepeta_subsp_spruneri	1990	Others	H	5	7	5	3	9	3	0	6
Coleostephus_myconis	1990	Stenomedit	T	8	9	4	3	5	4	0	6
Convolvulus_arvensis	1990	Eurasiat	G	7	7	5	4	5	5	0	6
Convolvulus_sepium	1990	Eurasiat	H	8	6	5	6	7	9	0	7
Crataegus_monogyna	1990	Eurasiat	P	6	7	5	4	6	3	0	4
Crepis_leontodontoides	1990	Others	H	5	8	4	4	3	7	0	3
Crepis_sancta_subsp_sancta	1990	Eurimedit	T	11	9	6	2	X	2	0	6
Crepis_vesicaria	1990	Eurimedit	T	8	8	3	3	6	2	0	6
Crithmum_maritimum	1990	Eurimedit	Ch	11	8	2	1	X	1	3	3
Cuscuta_cesattiana	1990	Naturalised	T	8	7	5	X	X	X	0	6
Cyclamen_hederifolium	1990	Stenomedit	G	4	8	5	5	5	5	0	1
Cyclamen_repandum_subsp_repandum	1990	Stenomedit	G	4	9	5	3	X	5	0	1
Cymbalaria_muralis	1990	Eurimedit	H	7	7	5	2	5	3	0	5
Cynodon_dactylon	1990	Cosmopol	G	8	8	5	4	X	4	0	7
Cynosurus_cristatus	1990	Eurasiat	H	8	5	4	5	5	4	0	6
Cynosurus_echinatus	1990	Eurimedit	T	11	9	5	2	4	2	0	5
Cyperus_longus_	1990	Eurasiat	G	8	7	5	11	5	5	0	7
Dactylis_glomerata_subsp_glomerata	1990	Eurasiat	H	7	6	5	4	5	6	0	5
Dasypyrum_villosum	1990	Eurimedit	T	8	10	5	2	4	2	0	6
Daucus_carota_subsp_carota	1990	Eurasiat	H	8	6	5	4	5	4	0	6
Dianthus_armeria_subsp_armeria	1990	Eurasiat	H	8	6	5	3	3	2	0	4
Digitaria_sanguinalis	1990	Cosmopol	T	7	7	5	3	6	4	0	7
Dioscorea_communis	1990	Eurimedit	G	5	7	5	5	8	6	0	1
Diplotaxis_erucoides_subsp_erucoides	1990	Stenomedit	T	8	8	4	3	5	5	0	6
Diplotaxis_tenuifolia	1990	Atlant	H	8	7	5	4	6	5	0	6
Dittrichia_graveolens	1990	Eurimedit	T	11	8	6	3	7	7	1	6
Dittrichia_viscosa_subsp_viscosa	1990	Eurimedit	H	11	8	5	3	7	9	0	6
Ecballium_elaterium	1990	Eurimedit	G	7	8	5	3	5	3	0	6
Echium_italicum_subsp_italicum	1990	Eurimedit	H	11	8	5	3	3	4	0	6
Echium_plantagineum	1990	Eurimedit	T	11	8	5	3	5	5	0	6
Eleocharis_palustris_subsp_palustris	1990	Cosmopol	G	8	6	5	1	3	3	0	6
Elymus_repens_subsp_repens	1990	Circumbor	G	7	X	7	5	X	8	0	6
Equisetum_ramosissimum	1990	Circumbor	G	7	7	6	3	7	1	0	6
Erica_arborea	1990	Stenomedit	P	6	8	4	3	2	1	0	3
Erigeron_bonariensis	1990	Naturalised	T	8	8	5	3	X	7	0	7
Erigeron_canadensis	1990	Naturalised	T	8	6	5	5	X	7	0	7
Erigeron_sumatrensis	1990	Naturalised	T	8	8	5	3	X	7	0	7
Erodium_acaule	1990	Others	H	11	8	3	3	3	3	0	6
Erodium_cicutarium	1990	Cosmopol	H	8	7	5	3	5	3	0	6
Erodium_malacoides_subsp_malacoides	1990	Stenomedit	T	11	9	4	2	5	2	0	6
Erodium_moschatum	1990	Eurimedit	T	11	9	5	2	5	2	0	7
Ervum_gracile	1990	Eurimedit	T	7	8	5	4	4	4	0	5
Eryngium_campestre	1990	Eurimedit	H	9	7	5	3	8	3	0	4
Euonymus_europaeus	1990	Eurasiat	P	6	5	5	5	8	5	0	2
Euphorbia_amygdaloides	1990	Eurasiat	Ch	4	5	4	5	7	6	0	2
Euphorbia_cuneifolia	1990	Stenomedit	T	7	7	4	6	7	4	0	5
Euphorbia_helioscopia_subsp_helioscopia	1990	Cosmopol	T	9	7	5	3	5	6	0	6
Euphorbia_peplus	1990	Circumbor	T	6	7	4	4	5	7	0	6

Euphorbia_prostrata	1990	Naturalised	T	7	8	5	2	5	4	0	7
Festuca_danthonii_subsp_danthonii	1990	Eurimedit	T	8	9	5	2	4	2	0	6
Festuca_ligustica	1990	Stenomedit	T	8	9	4	2	4	2	0	6
Festuca_myuros	1990	Cosmopol	T	8	9	5	2	6	2	0	6
Ficaria_verna_subsp_verna	1990	Eurasiat	G	4	5	5	6	7	7	0	3
Ficus_carica	1990	Eurimedit	P	7	8	6	X	5	X	0	3
Filago_germanica	1990	Eurasiat	T	8	7	5	3	4	2	0	5
Foeniculum_vulgare_subsp_piperitum	1990	Eurimedit	H	9	8	5	3	7	7	0	6
Fraxinus_angustifolia_subsp_oxycarpa	1990	Eurasiat	P	4	8	6	7	7	8	0	2
Fraxinus_ornus_subsp_ornus	1990	Eurasiat	P	5	8	6	3	8	3	0	3
Fumaria_capreolata_subsp_capreolata	1990	Eurimedit	T	7	9	5	3	5	3	0	5
Fumaria_officinalis	1990	Eurasiat	T	7	7	5	4	5	6	0	6
Galactites_tomentosus	1990	Stenomedit	H	8	8	4	3	X	7	0	6
Galatella_linosyris_subsp_linosyris	1990	Eurasiat	H	8	7	5	3	8	2	0	4
Galega_officinalis	1990	Eurasiat	H	7	8	7	6	5	6	0	6
Galium_aparine	1990	Eurasiat	T	6	X	5	4	5	5	0	4
Galium_palustre_subsp_elongatum	1990	Eurimedit	H	7	5	5	8	5	3	0	7
Gastridium_ventricosum	1990	Stenomedit	T	8	9	4	2	4	2	0	5
Gaudinia_fragilis	1990	Eurimedit	T	8	8	5	3	5	3	0	6
Geranium_columbinum	1990	Eurasiat	T	7	9	6	2	5	2	0	4
Geranium_dissectum	1990	Eurasiat	T	7	8	5	2	5	2	0	6
Geranium_molle	1990	Eurasiat	T	7	6	5	3	5	4	0	6
Geranium_purpureum	1990	Cosmopol	T	4	6	5	4	5	5	0	3
Geranium_rotundifolium	1990	Eurasiat	T	7	8	5	3	6	3	0	6
Glaucium_flavum	1990	Eurimedit	H	11	9	5	1	4	1	1	3
Hedera_helix_subsp_helix	1990	Eurimedit	P	4	5	4	5	X	X	0	1
Hedypnois_rhagadioloides	1990	Stenomedit	T	9	10	4	2	2	1	0	5
Heliotropium_europaeum	1990	Eurimedit	T	11	8	5	3	7	2	1	7
Helminthotheca_echioides	1990	Eurimedit	T	11	8	5	2	X	2	0	6
Helosciadium_nodiflorum_subsp_nodiflorum	1990	Eurimedit	H	7	8	5	10	X	6	0	8
Herniaria_hirsuta	1990	Eurasiat	T	9	6	5	4	2	2	0	6
Holcus_lanatus_subsp_lanatus	1990	Circumbor	H	7	5	4	6	X	4	0	6
Hordeum_bulbosum	1990	Cosmopol	H	8	10	5	4	5	4	0	6
Hordeum_murinum_subsp_leporinum	1990	Eurimedit	T	9	9	5	3	5	3	0	7
Hydrocotile_ranunculoides	1990	Cosmopol	G	9	8	X	9	4	3	0	
Hymenocarpos_circinnatus	1990	Stenomedit	H	11	9	4	2	2	2	0	5
Hyoseris_radiata	1990	Stenomedit	H	11	8	4	2	7	1	0	3
Hypericum_australe	1990	Stenomedit	H	7	8	4	7	6	4	0	4
Hypericum_perforatum_subsp_veronense	1990	Eurasiat	H	7	8	6	X	X	X	0	5
Hypericum_tetrapterum	1990	Eurasiat	H	7	7	6	4	4	4	0	6
Hypochoeris_achyrophorus	1990	Stenomedit	T	11	9	4	2	X	2	0	5
Hypochoeris_radicata	1990	Eurasiat	H	9	8	4	2	X	1	0	6
Isoetes_duriei	1990	Stenomedit	G	7	9	4	10	1	1	0	8
Isoetes_histrix	1990	Stenomedit	G	7	10	4	10	1	1	0	7
Jacobaea_erratica	1990	Eurasiat	H	7	6	4	4	7	4	0	6
Juncus_articulatus_subsp_articulatus	1990	Circumbor	G	8	7	4	8	6	5	0	6
Juncus_bufonius	1990	Cosmopol	T	4	7	5	6	4	1	0	7
Juncus_capitatus	1990	Eurimedit	T	8	10	2	8	4	1	1	7
Juncus_conglomeratus	1990	Circumbor	H	7	7	4	8	6	5	0	6
Juncus_effusus_subsp_effusus	1990	Cosmopol	H	7	7	5	9	6	5	0	6
Juncus_gerardi_subsp_gerardi	1990	Circumbor	G	8	6	4	5	7	5	2	4
Juncus_heterophyllus	1990	Atlant	I	7	7	3	10	4	3	0	3
Juncus_hybridus	1990	Eurimedit	T	8	8	3	8	6	3	0	7
Juncus_inflexus_subsp_inflexus	1990	Stenomedit	H	6	8	3	9	6	5	0	2
Juncus_subnodulosus	1990	Eurasiat	G	8	6	4	9	6	5	0	6
Kickxia_commutata_subsp_commutata	1990	Stenomedit	H	8	7	4	4	5	4	0	6
Kickxia_elatine_subsp_elatine	1990	Eurimedit	T	8	7	5	4	5	4	0	6
Knautia_integrifolia_subsp_integrifolia	1990	Eurimedit	T	7	8	5	3	3	2	0	6
Lagurus_ovatus_subsp_ovatus	1990	Eurimedit	T	8	9	5	3	X	2	1	5
Lamium_amplexicaule	1990	Eurasiat	T	7	7	5	4	5	7	0	7
Lamium_purpureum	1990	Eurasiat	T	7	7	5	4	5	5	0	7
Lathyrus_annuus	1990	Eurimedit	T	8	8	5	3	5	2	0	6
Lathyrus_aphaca_subsp_aphaca	1990	Eurimedit	T	6	6	5	3	X	X	0	3
Lathyrus_ochrus	1990	Stenomedit	T	7	8	4	2	5	2	0	6
Lathyrus_sphaericus	1990	Eurimedit	T	11	9	5	2	5	2	0	4
Laurus_nobilis	1990	Stenomedit	P	2	7	4	8	4	6	0	4

Lepidium_graminifolium_subsp_graminifolium	1990	Eurimedit	H	8	8	5	3	X	3	0	7
Ligustrum_vulgare	1990	Eurasiat	NP	7	6	4	X	8	X	0	3
Limbarda_crithmoides	1990	Stenomedit	Ch	11	8	4	7	9	5	3	5
Linum_corymbulosum	1990	Stenomedit	T	11	9	4	2	5	2	0	3
Linum_strictum	1990	Stenomedit	T	11	9	4	2	5	2	0	4
Linum_usitatissimum_subsp_angustifolium	1990	Eurimedit	H	7	7	5	3	7	2	0	6
Lipandra_polysperma	1990	Circumbor	T	6	5	5	6	4	8	0	7
Lolium_arundinaceum_subsp_arundinaceum	1990	Eurasiat	H	9	8	5	6	8	6	0	6
Lolium_multiflorum	1990	Eurimedit	T	7	7	5	4	X	6	0	6
Lolium_perenne	1990	Circumbor	H	8	5	4	5	X	7	0	6
Loncomelos_narbonense	1990	Eurimedit	G	8	7	5	4	6	4	0	5
Lonicera_caprifolium	1990	Eurasiat	P	6	5	6	6	X	5	0	1
Lotus_angustissimus	1990	Eurimedit	T	11	8	5	7	7	4	0	6
Lotus_corniculatus	1990	Eurasiat	H	7	X	5	4	7	2	0	4
Lotus_ornithopodioides	1990	Stenomedit	T	11	9	4	2	1	1	0	5
Lotus_tenuis	1990	Eurasiat	H	9	7	5	6	7	7	0	6
Lupinus_angustifolius	1990	Stenomedit	T	11	9	4	2	2	2	0	6
Luzula_forsteri	1990	Eurimedit	H	4	7	5	4	4	5	0	2
Lychnis_flos-cuculi	1990	Circumbor	H	7	5	4	6	X	6	0	3
Lysimachia_arvensis	1990	Eurimedit	T	6	6	5	5	X	6	0	6
Lysimachia_nardii	1990	Stenomedit	T	7	8	4	5	2	1	0	6
Lythrum_junceum	1990	Stenomedit	H	7	8	4	7	3	4	0	7
Lythrum_salicaria	1990	Cosmopol	H	7	5	5	8	7	X	0	7
Lythrum_tribracteatum	1990	Eurimedit	T	7	8	5	7	3	4	0	5
Malope_malacoides	1990	Stenomedit	T	9	9	5	2	5	4	0	6
Malva_punctata	1990	Stenomedit	T	8	9	4	2	5	4	0	6
Malva_sylvestris	1990	Circumbor	H	8	6	4	4	X	8	0	3
Matthiola_incana_subsp_incana	1990	Stenomedit	Ch	12	10	4	2	7	1	2	3
Medicago_arabica	1990	Eurimedit	T	9	9	5	2	X	2	0	7
Medicago_lupulina	1990	Eurasiat	T	7	5	X	4	8	7	0	6
Medicago_murex	1990	Stenomedit	T	11	9	4	2	X	2	0	6
Medicago_orbicularis	1990	Eurimedit	T	7	8	5	3	4	4	0	6
Medicago_polymorpha	1990	Eurimedit	T	9	9	5	2	X	2	0	6
Medicago_rigidula	1990	Eurimedit	T	11	8	5	1	X	1	2	5
Medicago_truncatula	1990	Stenomedit	T	11	8	4	1	X	1	2	5
Melica_minuta_subsp_latifolia	1990	Stenomedit	H	8	10	4	2	5	2	0	2
Melica_uniflora	1990	Eurasiat	H	3	5	5	5	6	X	0	1
Melissa_officinalis_subsp_altissima	1990	Eurimedit	H	6	7	5	4	6	4	0	6
Mentha_aquatica_subsp_aquatica	1990	Eurasiat	H	7	5	5	9	7	4	0	7
Mentha_pulegium_subsp_pulegium	1990	Eurimedit	H	8	7	5	7	X	2	0	6
Mentha_suaveolens_subsp_suaveolens	1990	Eurimedit	H	7	8	5	8	7	6	0	6
Mercurialis_annua	1990	Eurasiat	T	7	7	5	4	7	8	0	6
Muscari_comosum	1990	Eurimedit	G	7	8	5	3	7	0	0	4
Myosotis_ramosissima_subsp_ramosissima	1990	Eurasiat	T	9	8	5	2	4	3	0	6
Myrtus_communis	1990	Stenomedit	P	8	9	4	3	5	2	0	2
Narcissus_tazetta_subsp_tazetta	1990	Stenomedit	G	8	8	4	4	5	4	0	7
Nasturtium_officinale	1990	Cosmopol	H	7	4	5	11	7	7	0	7
Nigella_damascena	1990	Eurimedit	T	8	9	5	3	4	2	0	5
Oenanthe_fistulosa	1990	Eurasiat	H	7	7	5	9	7	5	0	6
Oenanthe_pimpinelloides	1990	Eurimedit	H	5	7	3	4	5	4	0	3
Oloptum_thomasii	1990	Eurasiat	H	5	7	4	4	7	5	0	5
Ononis_spinosa_subsp_antiquorum	1990	Eurimedit	T	8	6	5	X	X	3	0	5
Ophrys_apifera	1990	Eurimedit	G	7	6	5	4	9	2	0	4
Ophrys_bombyliflora	1990	Stenomedit	G	8	9	4	3	6	3	0	
Ophrys_sphegodes_subsp_sphegodes	1990	Eurimedit	G	8	8	5	4	9	3	0	4
Ophrys_tenthredinifera	1990	Stenomedit	G	8	9	4	3	6	3	0	0
Ophrys_x_sommieri	1990	Stenomedit	G								
Ornithopus_compressus	1990	Eurimedit	T	11	9	5	2	2	1	0	6
Orobanche_hederae	1990	Eurimedit	T	6	7	5	4	5	5	0	1
Oxalis_corniculata	1990	Eurimedit	H	7	7	0	4	X	6	0	7
Oxalis_dillenii	1990	Naturalised	H	7	7	5	5	5	7	0	7
Oxybasis_urbica	1990	Circumbor	T	8	7	4	6	7	6	0	8
Paliurus_spina-christi	1990	Eurasiat	P	7	8	6	3	7	3	0	4
Pallenis_spinosa_subsp_spinosa	1990	Eurimedit	T	11	9	5	4	X	7	0	5
Pancratium_maritimum	1990	Stenomedit	G	11	10	3	1	X	1	0	2
Papaver_rhoeas_subsp_rhoeas	1990	Others	T	6	6	5	5	7	X	0	6

Parapholis_incurva	1990	Stenomedit	T	11	7	4	5	7	2	3	5
Parietaria_judaica	1990	Eurimedit	H	7	8	5	3	X	6	0	5
Paspalum_distichum	1990	Cosmopol	G	X	8	X	10	8	8	0	7
Petrorhagia_dubia	1990	Stenomedit	T	11	8	5	2	8	2	0	6
Phalaris_brachystachys	1990	Stenomedit	T	7	7	X	5	6	4	0	6
Phalaris_truncata	1990	Eurimedit	H	7	7	X	4	6	4	0	6
Phillyrea_angustifolia	1990	Stenomedit	P	11	10	4	1	X	2	0	2
Phillyrea_latifolia	1990	Stenomedit	P	5	8	4	4	X	5	0	4
Phleum_nodosum	1990	Eurimedit	H	7	6	5	4	X	4	0	6
Phleum_pratense_subsp_pratense	1990	Circumbor	H	7	6	5	5	6	6	0	6
Phleum_subulatum_subsp_subulatum	1990	Stenomedit	T	8	3	4	5	8	7	0	4
Phragmites_australis	1990	Cosmopol	He	7	5	X	10	7	5	1	6
Picris_hieracioides	1990	Circumbor	H	8	X	5	4	8	4	0	6
Pinus_halepensis	1990	Stenomedit	P	11	10	4	2	0	2	0	1
Pinus_pinea	1990	Eurimedit	P	11	8	5	2	4	3	0	2
Pistacia_lentiscus	1990	Stenomedit	P	11	10	5	2	X	2	0	2
Pittosporum_tobira	1990	Naturalised	P	10	9	5	2	X	2	0	0
Plantago_coronopus	1990	Eurimedit	T	8	7	5	7	7	4	0	6
Plantago_crassifolia	1990	Stenomedit	H	11	8	4	3	9	4	1	3
Plantago_lanceolata	1990	Eurasiat	H	6	7	5	X	X	X	0	6
Plantago_macrorhiza	1990	Stenomedit	H	11	10	4	3	9	2	1	4
Plantago_major	1990	Eurasiat	H	8	X	X	5	X	7	0	7
Plantago_weldenii	1990	Eurimedit	T	8	7	5	7	7	4	0	4
Poa_annua	1990	Cosmopol	T	7	X	5	6	X	8	0	8
Poa_bulbosa	1990	Eurasiat	H	8	8	7	2	4	1	0	6
Poa_trivialis	1990	Eurasiat	H	6	X	5	7	X	7	0	6
Polycarpon_tetraphyllum_subsp_tetraphyllum	1990	Eurimedit	T	7	7	5	4	5	6	0	8
Polygonum_arenastrum	1990	Cosmopol	T	7	8	5	3	6	1	0	7
Polygonum_aviculare_subsp_aviculare	1990	Cosmopol	T	7	7	5	3	6	1	0	7
Polygonum_romanum	1990	Others	Ch	11	10	4	2	2	2	0	7
Polypodium_cambricum	1990	Eurimedit	H	3	8	5	3	X	5	0	2
Portulaca_oleracea	1990	Cosmopol	T	7	8	5	4	7	7	0	7
Potamogeton_nodosus	1990	Cosmopol	I	6	6	5	12	7	6	0	9
Potentilla_reptans	1990	Eurasiat	H	6	6	5	6	7	5	0	6
Poterium_sanguisorba_subsp_balearicum	1990	Eurasiat	H	7	6	5	3	8	2	0	4
Prospero_autumnale	1990	Eurimedit	G	8	8	4	2	6	3	0	5
Prunella_vulgaris_subsp_vulgaris	1990	Circumbor	H	7	6	4	6	4	X	0	3
Prunus_spinosa_subsp_spinosa	1990	Eurasiat	P	7	5	5	X	X	X	0	4
Pteridium_aquilinum_subsp_aquilinum	1990	Cosmopol	G	6	5	4	6	3	3	0	3
Pulicaria_dysenterica	1990	Eurimedit	H	8	6	5	7	X	5	0	7
Pyracantha_coccinea	1990	Stenomedit	P	5	8	4	3	5	3	0	6
Pyrus_communis_subsp_pyraster	1990	Eurasiat	P	6	5	5	6	7	7	0	5
Quercus_cerris	1990	Eurimedit	P	6	8	5	4	4	4	0	2
Quercus_ilex	1990	Stenomedit	P	2	9	4	3	X	X	0	2
Quercus_pubescens_subsp_pubescens	1990	Eurasiat	P	7	8	6	3	7	4	0	2
Quercus_robur	1990	Eurasiat	P	7	6	6	6	5	6	0	2
Ranunculus_bulbosus	1990	Eurasiat	H	8	6	5	3	7	3	0	6
Ranunculus_ophioglossifolius	1990	Eurimedit	T	7	7	5	8	6	6	0	7
Ranunculus_sardous	1990	Eurimedit	T	8	7	5	8	X	7	0	7
Ranunculus_velutinus	1990	Eurimedit	H	6	8	5	5	6	5	0	6
Raphanus_raphanistrum_subsp_raphanistrum	1990	Eurimedit	T	11	5	5	X	4	5	0	6
Reichardia_picroides	1990	Stenomedit	H	7	8	4	3	6	2	0	4
Rhamnus_alaternus_subsp_alaternus	1990	Stenomedit	P	4	9	5	2	4	4	0	3
Robinia_pseudoacacia	1990	Naturalised	P	5	7	5	4	X	8	0	6
Romulea_bulbocodium	1990	Stenomedit	G	8	9	4	3	4	3	0	5
Romulea_rollii	1990	Stenomedit	G	9	9	3	3	5	2	0	4
Rosa_sempervirens	1990	Stenomedit	NP	6	8	4	3	4	6	0	2
Rostraria_cristata	1990	Cosmopol	T	7	5	5	6	8	2	0	6
Rostraria_pubescens	1990	Stenomedit	T	7	8	4	5	8	2	0	4
Rubia_peregrina	1990	Stenomedit	P	5	9	4	4	5	3	0	1
Rubus_caesius	1990	Eurasiat	NP	7	5	5	7	7	9	0	3
Rubus_ulmifolius	1990	Eurimedit	NP	5	8	5	4	5	8	0	3
Rumex_acetosella_subsp_pyrenaicus	1990	Cosmopol	H	8	5	5	5	1	2	0	6
Rumex_bucephalophorus	1990	Eurimedit	T	8	12	5	2	2	1	0	6
Rumex_conglomeratus	1990	Eurasiat	H	8	7	5	7	X	8	0	6
Rumex_crispus	1990	Cosmopol	H	7	5	5	6	X	5	0	7

Rumex_obtusifolius	1990	Eurasiat	H	7	5	6	3	X	9	0	6
Rumex_pulcher_subsp_pulcher	1990	Eurimedit	H	8	8	5	2	6	9	0	7
Rumex_sanguineus	1990	Eurasiat	H	4	5	4	8	7	7	0	4
Ruscus_aculeatus	1990	Eurimedit	G	4	8	5	4	5	5	0	1
Sabulina_tenuifolia_subsp_tenuifolia	1990	Eurasiat	T	7	7	5	3	6	2	0	6
Sagina_apetala_subsp_apetala	1990	Eurimedit	T	8	7	5	6	4	5	0	8
Sagina_maritima	1990	Stenomedit	T	8	X	X	7	X	0	0	6
Salix_alba	1990	Eurasiat	P	5	6	6	7	8	7	0	7
Salsola_kali	1990	Eurasiat	T	9	7	8	8	7	8	2	3
Salvia_clandestina	1990	Eurasiat	H	8	8	6	3	5	7	0	6
Salvia_verbenaca	1990	Stenomedit	H	8	8	4	3	5	7	0	6
Sambucus_nigra	1990	Eurasiat	P	7	5	4	5	X	9	0	5
Schoenoplectus_lacustris	1990	Cosmopol	G	8	5	5	11	7	5	0	
Scirpoides_holoschoenus	1990	Eurimedit	G	8	8	5	8	5	4	0	4
Scorpiurus_muricatus	1990	Eurimedit	T	7	8	5	2	X	2	0	4
Scorzoneroides_cichoriacea	1990	Others	H	9	6	5	3	7	2	0	4
Sedum_cepaea	1990	Eurimedit	T	2	8	2	4	2	4	0	3
Senecio_vulgaris	1990	Eurimedit	T	7	X	X	5	X	8	0	7
Serapias_lingua	1990	Stenomedit	G	11	8	4	3	4	2	0	6
Serapias_parviflora	1990	Stenomedit	G	11	10	4	2	4	2	0	5
Serapias_vomeracea	1990	Eurimedit	G	11	8	5	3	4	2	0	4
Setaria_verticillata	1990	Cosmopol	T	7	8	5	4	X	8	0	7
Sherardia_arvensis	1990	Eurimedit	T	8	6	5	5	8	5	0	6
Silene_canescens	1990	Stenomedit	T	11	9	3	1	X	1	2	5
Silene_gallica	1990	Eurimedit	T	8	9	5	3	2	1	0	6
Silene_latifolia	1990	Stenomedit	H	6	9	4	3	4	2	0	3
Silene_vulgaris_subsp_tenoreana	1990	Eurasiat	H	8	X	X	4	7	2	0	5
Silybum_marianum	1990	Eurimedit	H	11	10	6	3	5	7	0	6
Sixalix_atropurpurea	1990	Stenomedit	H	6	8	4	3	X	2	0	5
Smilax_aspera	1990	Cosmopol	NP	6	10	4	2	5	3	0	1
Solanum_nigrum	1990	Cosmopol	T	7	6	5	3	5	7	0	7
Solanum_villosum	1990	Eurimedit	T	7	6	5	3	5	7	0	7
Sonchus_asper_subsp_asper	1990	Eurasiat	T	7	5	X	4	7	7	0	6
Sonchus_bulbosus_subsp_bulbosus	1990	Stenomedit	G	7	8	4	3	5	3	0	3
Sonchus_oleraceus	1990	Eurasiat	T	7	5	X	4	8	8	0	6
Sonchus_tenerrimus	1990	Stenomedit	T	7	8	4	2	5	4	0	6
Sorbus_domestica	1990	Eurimedit	P	4	7	5	3	8	3	0	1
Sorbus_torminalis	1990	Eurasiat	P	4	6	5	4	7	4	0	1
Sorghum_halepense	1990	Cosmopol	G	8	8	X	7	8	8	0	4
Spartium_junceum	1990	Eurimedit	P	7	7	5	4	7	2	0	4
Spergularia_marina	1990	Cosmopol	T	7	7	5	6	8	0	3	5
Spergularia_rubra	1990	Cosmopol	T	7	7	X	6	3	4	0	7
Spiranthes_spiralis	1990	Eurasiat	G	8	6	4	3	X	2	0	
Sporobolus_virginicus	1990	Cosmopol	G	11	11	4	1	0	1	3	3
Stachys_germanica_subsp_salviifolia	1990	Stenomedit	H	7	8	6	3	7	9	0	5
Stachys_ocymastrum	1990	Stenomedit	T	11	9	4	2	7	2	0	6
Stachys_romana	1990	Stenomedit	T	11	9	4	2	6	1	0	5
Stachys_sylvatica	1990	Circumbor	H	4	X	4	7	7	7	0	3
Stellaria_media	1990	Cosmopol	T	6	X	X	4	7	8	0	6
Stellaria_neglecta	1990	Eurasiat	T	6	7	5	4	5	8	0	4
Stellaria_pallida	1990	Eurasiat	T	8	8	5	3	5	4	0	7
Symphyotrichum_squamatum	1990	Naturalised	T	8	8	5	4	7	7	0	7
Thinopyrum_acutum	1990	Eurimedit	G	11	7	5	5	7	7	2	6
Thinopyrum_junceum	1990	Eurimedit	G	11	6	5	7	7	7	2	2
Thymelaea_passerina	1990	Eurimedit	T	8	7	5	3	7	2	0	5
Tordylium_apulum	1990	Stenomedit	T	11	9	4	2	X	2	0	6
Torilis_nodosa	1990	Eurimedit	T	7	8	6	4	7	6	0	6
Tribulus_terrestris	1990	Cosmopol	T	8	8	6	2	5	3	0	7
Trifolium_angustifolium_subsp_angustifolium	1990	Eurimedit	T	11	9	5	2	3	2	0	5
Trifolium_bocconei	1990	Stenomedit	T	7	7	4	4	7	2	0	6
Trifolium_campestre	1990	Eurasiat	T	8	5	5	4	X	3	0	6
Trifolium_fragiferum_subsp_fragiferum	1990	Eurasiat	H	8	6	5	7	8	7	0	6
Trifolium_glomeratum	1990	Eurimedit	T	7	7	5	3	2	2	0	6
Trifolium_ligusticum	1990	Stenomedit	T	8	9	4	2	2	1	0	6
Trifolium_nigrescens_subsp_nigrescens	1990	Eurimedit	T	8	6	5	5	5	6	0	6
Trifolium_pallidum	1990	Eurimedit	T	7	8	5	4	2	2	0	6

Trifolium_pratense_subsp_pratense	1990	Circumbor	H	7	X	4	X	X	X	0	6
Trifolium_repens	1990	Eurasiat	H	8	X	X	X	X	7	0	7
Trifolium_scabrum	1990	Eurimedit	T	11	8	5	2	9	1	0	5
Trifolium_stellatum	1990	Eurimedit	T	11	9	5	2	X	2	0	4
Trifolium_subterraneum	1990	Eurimedit	T	11	9	5	2	2	2	0	6
Triglochin_laxiflora	1990	Stenomedit	G	8	8	4	8	7	7	0	0
Triticum_vagans	1990	Stenomedit	T	11	10	X	5	5	4	0	4
Tuberaria_guttata	1990	Eurimedit	T	11	9	5	2	1	1	0	6
Typha_angustifolia	1990	Circumbor	G	8	7	5	10	X	7	0	6
Typha_latifolia	1990	Cosmopol	G	8	6	5	10	X	8	0	6
Ulmus_minor	1990	Eurasiat	P	5	7	5	X	8	X	0	4
Urospermum_dalechampii	1990	Eurimedit	H	8	8	5	3	X	3	0	4
Urtica_dioica	1990	Cosmopol	H	X	X	X	6	X	8	0	7
Urtica_membranacea	1990	Stenomedit	T	7	8	5	3	6	3	0	6
Valerianella_eriocarpa	1990	Stenomedit	T	11	9	4	2	5	1	0	6
Verbascum_blattaria	1990	Eurasiat	H	8	6	7	3	7	6	0	6
Verbascum_sinuatum	1990	Eurimedit	H	9	8	5	3	7	7	0	6
Verbena_officinalis	1990	Eurasiat	H	9	5	5	4	X	6	0	6
Veronica_arvensis	1990	Eurasiat	T	5	5	5	5	6	X	0	7
Veronica_beccabunga_subsp_beccabunga	1990	Eurasiat	H	7	X	5	10	7	6	0	7
Veronica_persica	1990	Naturalised	T	8	7	5	5	5	6	0	7
Veronica_serpyllifolia	1990	Eurasiat	H	X	X	5	3	5	X	0	3
Viburnum_tinus_subsp_tinus	1990	Stenomedit	P	5	9	4	4	5	3	0	2
Vicia_angustifolia	1990	Eurimedit	T	5	5	6	X	X	X	0	6
Vicia_benghalensis	1990	Stenomedit	T	11	9	4	2	5	2	0	7
Vicia_bithynica	1990	Eurimedit	T	7	7	5	3	5	5	0	5
Vicia_dasycarpa	1990	Eurimedit	T	7	6	5	4	4	5	0	6
Vicia_hybrida	1990	Eurimedit	T	7	8	5	3	5	5	0	6
Vicia_pseudocracca	1990	Eurimedit	T	7	6	5	4	4	5	0	5
Vinca_major_subsp_major	1990	Eurimedit	Ch	6	7	5	4	5	3	0	3
Viola_odorata	1990	Eurimedit	H	5	6	5	5	X	8	0	6
Viola_reichenbachiana	1990	Circumbor	H	4	5	4	5	7	6	0	2
Viola_suavis	1990	Eurasiat	H	5	8	6	5	4	4	0	2
Vitis_vinifera	1990	Cosmopol	P	6	8	5	6	8	6	0	2
Xanthium_italicum	1990	Eurimedit	T	8	8	5	5	X	1	0	6

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POSTHARVEST LONGEVITY AND PHYSIOLOGICAL CHANGES IN CUT ASPARAGUS PLUMOSUS FOLIAGE AS INFLUENCED BY PREHARVEST AND POSTHARVEST TREATMENT OF SALICYLIC ACID AND GIBBERELLIC ACID

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abstract – *Asparagus plumosus* have plenty of applications in floral decoration, but its postharvest longevity is short. So, the present research aimed to improve vase life and some physiological parameters of this plant species in a factorial experiment based on a randomized complete block design with 16 treatments and three replications. The experimental treatments included two plant growth regulators of gibberellic acid (GA_3) and salicylic acid (SA) at four rates (0, 100, 200, and 400 µmol) and two application methods of foliar application at the preharvest phase and application in the preservative solution at the postharvest phase. In both application methods, SA and GA_3 improved all traits versus the control irrespective of the application method. However, the combined application of 100 and 200 µmol of GA_3 and SA exhibited the best results and the longest vase life. The weakest results among different rates were obtained from increasing the application rate of SA and GA_3 to 400 µmol. In general, the best treatments for improving the vase life and related traits were SA100 + GA200 in the foliar application and SA200 + GA100 in the vase solution application. So, they are recommended for preserving the postharvest quality of this plant species.

Keywords: antioxidant enzymes, foliar application, growth regulators, malondialdehyde.

Introduction

The production and export of foliage plants alongside cut and pot flowers have had an ascending trend in flower-producing and exporting countries in recent years. Foliage flowers are a major element in floriculture, especially floral design and decoration. In addition to their beautifying role, these flowers are used as fillers in flower bouquets and baskets. The foliage flower most commonly used in floral design is *Asparagus plumosus*, which provides one of the most popular ornamental leaves in flower decoration. *A. plumosus* is a perennial herbaceous plant with strong green stalks and flat and feather-like leaves from the family of Asparagaceae that is mainly cultivated to produce ornamental leaves in floriculture (Safeena et al., 2014; Chowdhuri et al., 2021).
As with cut flowers, postharvest shelf life is a key factor in assessing cut foliage quality and commercial value. Ornamental foliage plants like *A. plumosus* immediately lose their ornamental value after detachment from their maternal plants due to leaf shedding, early withering, and/or leaf bleaching or browning (Safeena et al., 2014). So, extending postharvest longevity is necessary to preserve cut foliage ornamental and commercial value. Various processes are involved in accelerating the senescence of cut flowers and foliage, including the inability of the cut part to take up water due to the activity of microorganisms and ethylene sensitivity. Extensive research, especially on cut flowers,

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Table 1. The comparison of the mean effects of different treatments (pre-harvest) on the studied parameters of *Asparagus plumosus* *

Treatment	Total chlorophyll content (mg g^{-1} F.W.)	Electrolyte leakage (%)	Relative solution uptake (%)	Bacterial load (%)	Relative water content (%)	MDA (mMol g^{-1} F.W.)	CAT (units g^{-1} F.W. min^{-1})	SOD (units g^{-1} F.W. min^{-1})	POD (units g^{-1} F.W. min^{-1})
Control	1.53 f	36.76 a	49.50 f	7.29 a	60.17 i	52.95 a	1.17 e	1.25 e	0.78 j
GA_{100}	2.64 bc	19.37 de	81.45 a-d	1.94 cde	80.08 c	19.57 h	3.23 b	3.20 abc	2.17 de
GA_{200}	2.64 bc	18.78 f	80.82 a-d	1.92 cde	80.33 c	19.37 h	3.30 ab	3.21 abc	2.25 d
GA_{400}	2.44 de	20.38 bc	75.92 cde	1.94 cde	73.68 h	23.08 c	3.18 bc	2.98 bcd	1.36 i
SA_{100}	2.73 ab	19.14 e	81.62 abc	1.93 cde	79.99 c	18.28 i	3.29 ab	2.65 d	2.06 e
SA_{100}-GA_{100}	2.70 ab	18.66 fg	83.49 a	1.90 cde	81.88 b	18.68 i	3.30 ab	3.21 abc	2.54 c
SA_{100}-GA_{200}	2.86 a	17.26 i	83.58 a	1.88 e	82.66 ab	16.95 j	3.39 a	3.39 a	3.51 a
SA_{100}-GA_{400}	2.55 bcd	20.19 c	78.18 a-e	1.97 cd	74.13 gh	22.58 de	3.23 bc	3.09 abc	1.60 gh
SA_{200}	2.65 bc	18.71 f	73.88 e	1.95 cde	80.49 c	19.24 h	3.27 ab	3.16 abc	2.26 d
SA_{200}-GA_{100}	2.68 abc	18.36 g	82.51 ab	1.92 cde	81.91 b	18.25 i	3.37 a	3.32 ab	2.98 b
SA_{200}-GA_{200}	2.75 ab	17.73 h	83.77 a	1.89 de	83.03 a	17.13 j	3.39 a	3.32 ab	3.45 a
SA_{200}-GA_{400}	2.62 bcd	19.30 de	78.42 a-d	1.94 cde	78.33 d	21.37 f	3.22 bc	3.22 abc	1.83 f
SA_{400}	2.43 de	20.42 bc	76.59 b-e	1.98 c	74.99 fg	22.34 e	3.11 cd	3.17 abc	1.40 i
SA_{400}-GA_{100}	2.49 cde	20.26 bc	77.76 a-e	1.97 c	75.15 f	23.02 cd	3.21 bc	3.12 abc	1.69 fg
SA_{400}-GA_{200}	2.49 cde	19.50 d	78.11 a-e	1.94 cde	77.39 e	20.71 g	3.28 ab	3.16 abc	1.83 f
SA_{400}-GA_{400}	2.33 e	20.56 b	75.08 de	2.09 b	74.00 h	24.15 b	3.07 d	2.91 cd	1.45 hi

*In each column, averages that have at least one common letter are in statistically similar groups with Duncan's multiple range tests at the $P < 0.01$ level.

has revealed that SA and GA_3 are effective compounds in retarding senescence and preserving postharvest longevity (Alaey et al., 2011; Saeed et al., 2014; Abbasi et al., 2019). Salicylic acid (SA) is a naturally-occurring simple phenolic compound that is synthesized in plant cells and acts as a signaling molecule in preserving plant resistance to biotic and abiotic stresses, e.g., plant resistance to pathogens (Murphy et al., 2020). Although SA is recognized as a defensive hormone in plants, it plays a regulating role in all physiological processes of plants during their growth and development. SA is extensively applied as a safe and environmentally-friendly compound at preharvest and postharvest stages. Previous research has shown that SA plays an essential role in regulating and controlling the senescence process, scavenging free radicals, strengthening the antioxidant system, preserving membrane stability, and maintaining postharvest quality (Xiang et al., 2021; ul Haq et al., 2022). Furthermore, the antimicrobial activity of SA and its effects on stomatal closure, respiration suppression, ethylene synthesis, and the retardation of the postharvest aging trend have been documented (Alaey et al., 2011; ul Haq et al., 2022). The increase in vase life and its related traits with SA application has been reported in the cut flowers of roses (Alaey et al., 2011), iris (Ramzan et al., 2018), and lily (Abbasi et al., 2019), reflecting the effectiveness of the compound in preserving postharvest longevity. SA inhibits pathogen growth in plants through repression of the auxin signaling pathway, which causes the global repression of auxin-related genes, including the TIR1 receptor gene, resulting in the stabilization of the Aux/IAA repressor proteins and inhibition of auxin responses (Wang et al., 2007). SA prevents the conversion of ACC to ethylene by reducing the production and activity of ACC oxidase. SA was also found to inhibit the wound-induced accumulation of the ACC synthase transcript in tomato (Wei et al., 2011). According Wei et al. (2011) report, SA could be considered for commercial application to maintain the quality and antioxidant properties of asparagus. SA treatments may enhance cut flower longevity by promoting membrane stability and maintaining water uptake. SA is an acid that can function as signal molecules in promoting plant defence responses. SA and JA markedly stretched the vase life of cut *Acacia holosericea* foliage by inhibiting water loss and maintaining relative fresh weight during the vase life (Chen & Joyce, 2016).

Gibberellic acid (GA_3) is the most common gibberellic used in horticulture. This plant growth regulator participates in all physiological processes during plant growth and development, from germination to aging and even during storage and postharvest life. GA_3 is an anti-aging compound and prevents pigment degradation in plant tissues (Ferrante

Table 2. The comparison of the mean effects of different treatments (post-harvest) on the studied parameters of *Asparagus plumosus* *

Treatment	Total chlorophyll content (mg g^{-1} F.W.)	Electrolyte leakage (%)	Relative solution uptake (%)	Bacterial load (%)	Relative water content (%)	MDA (mMol g^{-1} F.W.)	CAT (units g^{-1} F.W. min^{-1})	SOD (units g^{-1} F.W. min^{-1})	POD (units g^{-1} F.W. min^{-1})
Control	1.24 f	37.85 a	48.22 j	7.76 a	59.35 h	54.17 a	1.34 e	1.35 g	0.92 l
GA_{100}	2.13 e	23.15 g	76.67 e	2.12 cd	74.35 e	21.18 f	2.74 d	3.13 bcd	2.28 fg
GA_{200}	2.27 b-e	21.75 i	80.05 c	2.08 cd	76.54 d	20.39 h	3.16 abc	3.21 ab	2.43 e
GA_{400}	2.20 cde	23.46 fg	74.06 h	2.15 cd	72.90 f	23.58 c	3.11 abc	3.02 de	1.55 k
SA_{100}	2.24 b-e	22.59 h	77.07 e	2.08 cd	74.97 e	21.32 f	3.11 abc	3.09 bcd	2.23 g
SA_{100}-GA_{100}	2.29 a-e	20.72 j	80.99 b	2.12 cd	77.40 c	19.97 i	3.33 ab	3.26 a	2.67 d
SA_{100}-GA_{200}	2.29 a-e	20.45 j	80.51 c	2.10 cd	77.89 c	19.78 ij	3.21 abc	3.21 ab	3.21 c
SA_{100}-GA_{400}	2.29 a-e	24.21 cd	75.28 g	2.15 cd	73.29 f	23.39 c	3.11 abc	3.05 de	1.74 j
SA_{200}	2.33 abc	20.68 j	79.45 d	2.12 cd	76.28 d	20.72 g	3.17 abc	3.20 abc	2.40 ef
SA_{200}-GA_{100}	2.44 a	19.67 k	81.93 a	1.99 d	80.76 a	19.56 j	3.32 ab	3.31 a	3.60 a
SA_{200}-GA_{200}	2.37 ab	19.58 k	81.05 b	2.00 d	79.98 b	19.81 ij	3.34 a	3.26 a	3.38 b
SA_{200}-GA_{400}	2.29 a-e	23.57 ef	75.97 f	2.12 cd	74.43 e	22.92 d	3.17 abc	3.08 cd	2.04 h
SA_{400}	2.25 b-e	24.44 bc	74.42 h	2.19 c	72.64 f	23.64 c	3.04 bc	3.05 de	1.60 k
SA_{400}-GA_{100}	2.25 b-e	23.88 de	75.92 f	2.21 c	73.02 f	22.78 d	3.01 c	2.95 e	1.90 i
SA_{400}-GA_{200}	2.32 a-d	24.24 cd	75.83 f	2.24 c	74.76 e	22.39 e	3.10 abc	3.10 bcd	2.00 hi
SA_{400}-GA_{400}	2.17 de	24.71 b	73.42 i	2.66 b	71.00 g	24.66 b	2.97 cd	2.69 f	1.64 jk

*In each column, averages that have at least one common letter are in statistically similar groups with Duncan's multiple range tests at the $P < 0.01$ level.

et al., 2009; Miceli et al., 2019; Xiao et al., 2022). In Zhang et al.'s (2022) research, the postharvest application of GA_3 contributed to preserving growth, chlorophyll content, the activity of antioxidant enzymes, and postharvest quality and longevity of lilies. Saeed et al. (2014) reported that the foliar treatment of gladiolus with 25 mg/l GA_3 resulted in preserving membrane stability and antioxidant activity and extending vase life. The positive effects of GA_3 have also been reported on the longevity and postharvest quality of lettuce and *Eruca sativa* (Miceli et al., 2019) and okras (Xiao et al., 2022). Experiment was done to assess the effects of GA (0.25 mmol·dm^{-3} GA_3) as well as the Dutch commercial conditioners on the senescence of cut foliage of *Asparagus densiflorus* 'Meyerii'. An growth in the proteolytic activity was detected during shoot senescence with simultaneous reduction in soluble proteins and accumulation of free amino acids and proline. Postharvest treatments significantly impacted the rate of the above alterations (Rabiza-Świder & Skutnik, 2009; Safeena, 2013). The results of Rabiza-Świder et al. (2009) study clearly indicate that GA_3 or Chrysal SVB® can perform their positive effects in lengthening the vase life of cut *A. densiflorus* 'Myriocladus' shoots, by delaying some senescence-related activities in their cladodes.

Given the significance of *A. plumosus* as one of the most widely used ornamental foliage in floral design and the positive effect of GA_3 and SA in extending the longevity of perishable horticultural products, the present research aimed to shed light on the effect of these two growth regulators at the preharvest and postharvest stages on the vase life and related traits of cut *A. plumosus* foliage. This test surveyed potentially beneficial effects of GA_3 and SA vase solution treatments on vase life reactions and longevity of cut A. plumosus foliage.

Materials and Methods

To study the effect of growth regulators on the postharvest longevity of cut *Asparagus plumosus* foliage, a factorial experiment was conducted based on a randomized complete block design with three replications. The experimental treatments included two growth regulators (GA_3 and SA) at four rates (0, 100, 200, and 400 µmol) and two application times (preharvest as foliar application and postharvest in vase solution). For the experiment, 480 pot plants of *A. plumosus* that were similar in age, size, and maturity level were procured from a commercial greenhouse in Amol, Mazandaran and were immediately transferred to

a greenhouse with standard and uniform conditions for all plants (24±2°C and 60-70% relative humidity) and were used as the plant material. The experiment was carried out in two phases (preharvest and postharvest). In the first phase, 240 plants were sprayed with different rates of SA and GA_3 two weeks before commercial maturity. The plants were sprayed only once with 500 ml solution/plant so that the surface of all the plants was wet after the foliar application and the solution drops were flowing on their leaves. Two weeks after the foliar application (at the commercial maturity stage), one branch was detached from each plant and sent to the postharvest laboratory of Science and Research Branch of Islamic Azad University for the study of postharvest longevity. At the laboratory, all branches were cut to the same length, and after weighing, they were re-cut under water to avoid vascular blockage. They were then put in vases containing 250 ml of the vase solution (water + sucrose 3% + 8-hydroxyquinoline sulfate) and were kept there until the end of their vase life. The vases were kept in a room at a temperature of 20±2°C, relative humidity of 70-75%, and 12 hours of daylight with a light intensity of 15 µmol/m^2/s throughout the experiment.

In the second phase, the 240 *A. plumosus* plants, which had not been sprayed at the preharvest stage, were used. The branches were cut from their material plants at the commercial maturity stage and were transferred to the laboratory as soon as possible. At this stage, different rates of GA_3 and SA were used as the vase solution and distilled water was used as control. The procedure for preparing and keeping the cut foliage for the assessment of postharvest parameters was similar to the first phase.

The flowers were daily visited to register the target parameters. The vase life was calculated by counting days from foliage harvest from the material plants until the shedding and withering of 30% of the foliage. Vase life was recorded in days. The end of vase life was defined as the point when 30% of the shoots showed signs of yellowing or drying out (Skutnik et al., 2006).

Some other recorded traits included relative uptake of solution, relative water content (Sedaghathoor et al., 2020), bacterial load (Knee, 2000), total chlorophyll (Arnon, 1949), electrolyte leakage (Zhao et al., 1992), malondialdehyde (MDA) content (Stewart & Bewley, 1980), and the activity of catalase, superoxide dismutase, and peroxidase (Mazumdar & Majumder, 2017). To measure the water content of the petals, they were weighed on the first and final day of the experiment and their fresh weight was recorded. Then, they were oven-dried at 60°C for 72 hours and their dry weight was recorded. The following equation was employed to measure the rate of solution uptake (Sedaghathoor et al., 2020).

$$\text{Solution uptake rate} = \frac{\text{Daily weight of foliage - weight on 1 st day}}{\text{Weight on 1 st}} \times 100$$

The activity of catalase was measured through the following stages (Mazumdar & Majumder, 2017): 1 g of plant tissue that had been ground in 4 ml ethanol was added with (i) 0.01 mol phosphate buffer (pH = 7), (ii) 0.5 ml H2O2 0.2 mol, and (iii) 2 ml acid reagent (dichromate/acetic acid mixture). Then, its absorption was read at 610 nm with a spectrophotometer. To measure the enzymatic activity of peroxidase (POD), the extract was prepared as described

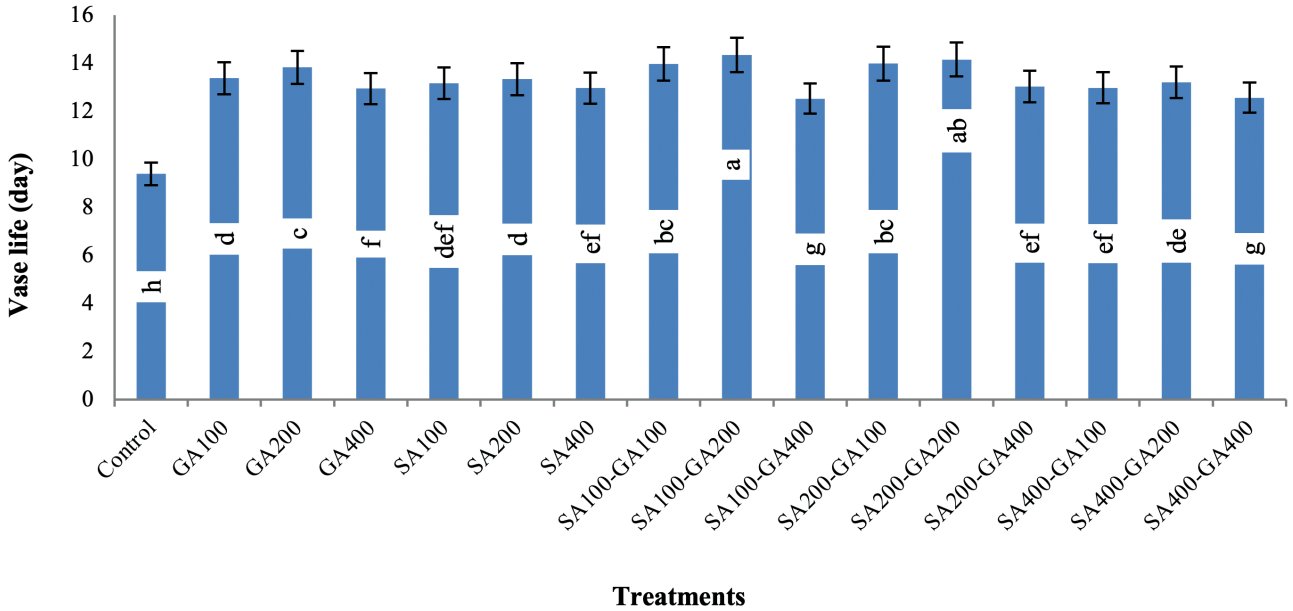

Figure 1. The effect of the foliar application of SA and GA_3 at different rates on the vase life of *Asparagus plumosus.*

above. Then, the variations of OD were read at 430 nm with a spectrophotometer once thirty seconds for two minutes (Mazumdar and Majumder, 2017). At the end of the experiment and after data collection, they were subjected to variance analysis (ANOVA) in MSTATC and the comparison of means by Tukey test at the 1% and 5% probability levels.

Results

The comparison of means for the effect of the preharvest foliar application of growth regulators on the recorded traits of *A. plumosus* revealed that the application of SA and GA_3, separately and together, significantly increased total chlorophyll content, relative solution uptake, relative water content, and the activity of antioxidant enzymes. Also, the preharvest foliar application of SA and GA_3 was significantly related to lower bacterial load, electrolyte leakage (EL), and MDA accumulation than the control (Table 1). The comparison of means for the application of SA and GA_3 at the preharvest step showed the positive and significant effect of their application, both separately and together, on extending the vase life of *A. plumosus* so that the control had the shortest vase life (9.40 days) and SA_{100} + GA_{200} had the longest vase life (14.35 days). SA_{200} + GA_{200} exhibited the second-longest vase life (Figure 1). According to the results, the most effective treatments in extending the vase life and improving the recorded parameters were SA_{100} + GA_{200} and SA_{200} + GA_{200}. Based on Table 1, SA_{100} + GA_{200} treatment resulted in the highest values of total chlorophyll, relative solution uptake, CAT and SOD activities. While this treatment caused to the lowest amount of bacterial load and electrolyte leakage. Thus, SA_{100} + GA_{200} was one of the effective treatments for *Asparagus plumosus* cut foliage. It can be concluded that the highest electrolyte leakage and MDA were related to the control treatment, which indicates in the pre-harvest experiment, positive effects were observed with the application of trial PGRs compared to the control (Table 1).

Table 2 presents the effect of SA and GA_3 application at the postharvest phase and as the vase solution on the physiological parameters of the cut *A. plumosus* foliage. Similar to the results of the preharvest phase, the application of SA and GA_3, either separately or combination, improved the recorded parameters versus the control significantly. According to the results, SA_{200} + GA_{100} and SA_{200}+ GA_{200} were the first and second-best treatments as they improved most recorded traits, respectively (Table 2). The comparison of means revealed that the application of SA and GA_3-containing vase solution extended the vase life versus the control (9.49 days). The lowest vase lives were obtained from the treatments of SA_{200} + GA_{100} (14.15 days), SA_{200} + GA_{200} (14.06 days), and SA_{100} + GA_{200} (14.02 days) (Figure 2).

But the interesting result is that in the post-harvest test, the aforementioned traits (i.e. EL, bacterial load and MDA) had the same results as the pre-harvest test under the control, so that the highest value of EL, bacterial load, and MDA were related to control the same as preharvest experiment. In the post-harvest experiment, the highest values of of total chlorophyll, relative solution uptake and CAT activities were

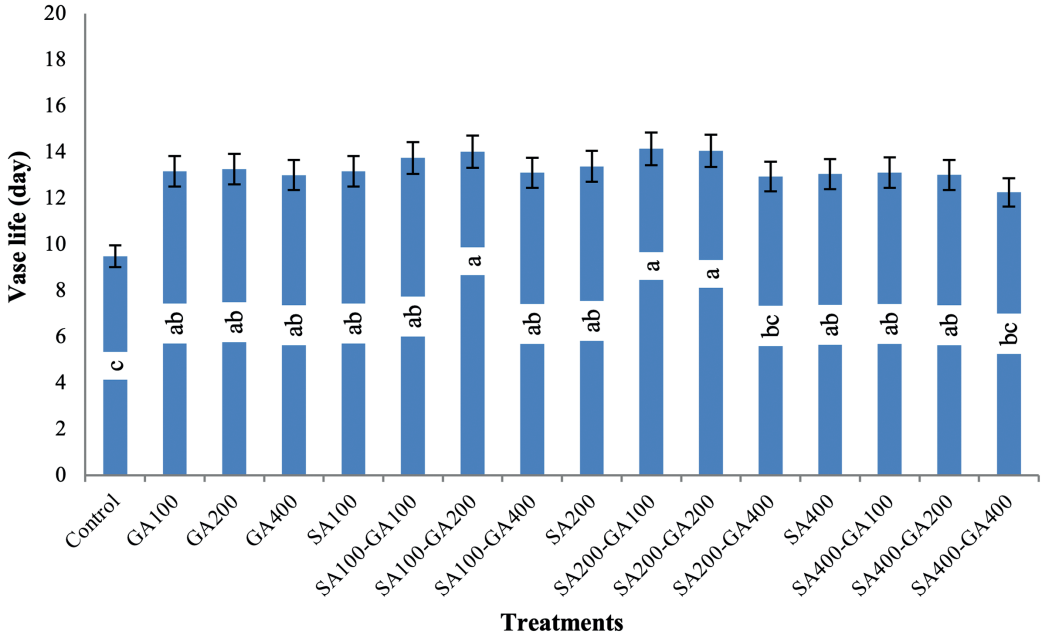

Figure 2. The effects of vase solution containing SA and GA_3 at different rates on the vase solution of *Asparagus plumosus*.

associated to combination treatment of SA + GA as well as pre-harvest test, but, in post harvest test, treatment SA_{200} + GA_{100} resulted in the highest value of total chlorophyll, relative solution uptake, CAT and SOD activities (Table 2). It seems that, unlike the pre-harvest test, the high concentration of SA (200) is more effective in the post-harvest test.

Discussion

Postharvest longevity is the most important qualitative parameter that determines the commercial value of all fresh and perishable products, like cut flowers and foliage. One of the most important factors that accelerate the aging and withering of the flowers and foliage detached from the maternal plants is the disruption in water uptake. Vascular blockage by microbial and bacterial factors is an essential impediment to water uptake by cut branches (Alaey et al., 2011). So, the elimination of bacteria from vase solutions and stem ends can contribute to improving vase life. In the present research, the separate and simultaneous application of SA and GA_3 at both preharvest and postharvest phases and at all rates reduced bacterial load versus the control, which was accompanied by an increase in solution uptake and relative water content. SA is an anti-stress compound that increases water uptake in plants by influencing the accumulation of compatible osmolytes (ul Haq et al., 2022). In the present research, the lowest solution uptake was observed in the control whereas the application of SA and GA_3, either separately or together, increased the solution uptake of the cut foliage by over 1.5 times. Since solution uptake by cut foliage is related to bacterial load, we observed that it was increased by reducing the bacterial load. Of course, different species and cultivars of *Asparagus* have different responses to water uptake. So that water uptake of cut 'Myriocladus' foliage was almost double that of cut *A. plumosus* foliage, perhaps due to different morphology of transpiration apparatus. (Marino et al., 2003). SA has anti-microbial activity (ul Haq et al., 2022), so its effect on reducing the microbial load is not surprising. On the other hand, it has been reported that SA results in stomatal closure in adverse conditions to reduce water loss by transpiration (ul Haq et al., 2022), It can, therefore, be said that SA helped preserve water uptake and even relative water content of the cut *A. plumosus* foliage by performing multiple functions, including the reduction of bacterial load and the reduction of transpiration. Similar results have been reported by Alaey et al. (2011) for cut roses and ul Haq et al. (2022) for cut *Consolida ajacis* spikes.

GA_3 plays a key role in making a balance in the water content of plant cells (Janowska et al., 2013; Ulczycka-Walorska & Krzymińska, 2022). Stephen et al. (2005) reported that GA_3 improved water uptake and relative water content in plant tissues by increasing cell wall flexibility. Mashahiri & Asil (2018) reported the increased uptake of water and relative water content in cut daffodil flowers with GA_3 application. They argue that GA_3 creates negative water potential in cells by facilitating the decomposition of complicated carbohydrates into simple sugars and their accumulation in cells, thereby enabling cells to take up more water and increase their relative water content. Similar results have been reported by Emongor (2004) for gerbera and Pinto et al. (2007) for *Calathea louisae*. In addition, GA_3 has been reported to reduce bacterial accumulation and inhibit vascular blockage (Singh et al., 2008). So, the preservation of relative water content in the cut *A. plumosus* foliage by the preharvest and postharvest application of SA and GA_3 can be ascribed to their impact on reducing bacterial load, decreasing cell water potential, preserving water uptake, and reducing water loss.

Yellowing or chlorophyll degradation in ornamental foliage plants and green parts of plants is a symptom of aging and the loss of postharvest commercial value (Supapvanich & Promyou, 2013; Xiao et al., 2022). One key problem of *A. plumosus* is the early yellowing of leaves at the postharvest stage (Safeena et al., 2014). As was already mentioned in the results, SA and GA_3 were beneficial for preserving leaf chlorophyll at both preharvest and postharvest stages. There are reports as to chlorophyll preservation in the cut foliage of *Clathea* (Pinto et al., 2007), citrus (Porat et al., 2001), and *Limonium latifolium* (Janowska et al., 2013) with GA_3 application. Porat et al. (2001) state that GA_3 application timing (preharvest and postharvest) does not affect chlorophyll significantly. We also observed that although chlorophyll content was higher when GA_3 and SA were applied at the postharvest phase, the difference was extremely slight. Rouhi et al. (2014) reported that GA_3 prevented pigment degradation and early withering by acidifying cell sap. Similarly, Stephen et al. (2005) attribute the positive effect of GA_3 on chlorophyll preservation to its structural role in chloroplast structure and its effect on photosynthesis. Ethylene is the main cause of chlorophyll degradation. So, the inhibition of its synthesis and activity can help chlorophyll preservation. SA is an ethylene suppressor and contributes to preserving chlorophyll by suppressing ethylene synthesis (Horváth et al., 2007; Supapvanich & Promyou, 2013). Wei et al. (2011) revealed that SA application (0.1 mmol/l) over six days of asparagus storage helped its chlorophyll preservation, but increasing its rate to 1 mmol/l had a negative effect on this trait. We found that the combined application of the highest rates of GA_3 and SA did not have an acceptable impact on chlorophyll preservation compared to their lower rates. In the study of Dolci et al. (1989), during vase life both species of *Asparagus* showed progressive senescence characterised

by yellowing and falling of cladodes. The vase life of cut *A. plumosus* ranged between 22 and 24 days.

The increased activity of reactive oxygen species (ROS), damages to membrane structure, electrolyte leakage, and MDA accumulation are the major indicators of aging initiation in plants (ul Haq et al., 2022). The excessive amount of ROS and its activity in cells cause oxidative stress, disrupt the physiological functioning of the plant, and injure membrane and macromolecules. Normally, plants scavenge ROS and maintain its balance in their tissues by increasing antioxidant activity. But, as aging proceeds, ROS synthesis and activity sharply increase, resulting in membrane degradation and finally, cell death (Shabanian et al., 2019; ul Haq et al., 2022). SA is an anti-stress hormone that builds a strong defense against oxidative stress by increasing the activity of antioxidant enzymes. By enhancing the activity of POD, SOD, CAT, and APX enzymes, SA helps scavenge ROS, protect the health and integrity of membrane structure, and prevent electrolyte leakage and MDA accumulation. Supapvanich & Promyou (2013) also reported the stimulated activity of antioxidant enzymes (POD, SOD, and CAT) and the reduction of lipid peroxidation by SA. Singh et al. (2008) found that GA_3 application in the vase solution of gladiolus contributed to sustaining antioxidant activity, preserving membrane structure, and delaying aging and death of petals. Researchers argue that GA_3 prevents cell sap acidification by decomposing proteins and degrading cell membrane in cut daffodil flowers (Mashahiri & Asil, 2018). The preservation of membrane stability, the reduction of MDA accumulation, and the increase in the activity of antioxidant enzymes have been reported in cut gladiolus (Saeed et al., 2011) and lily 'Caroline' flowers (Zhang et al., 2022) with GA_3 application, which is consistent with our results.

As is known, the occurrence and acceleration of aging at the postharvest stage depend on many environmental, physical, and chemical factors in addition to detachment from the material plant. These factors trigger destructive changes in biochemical and physiological processes in cut foliage. These changes are typically accompanied by a decrease in water uptake, an increase in water loss, a decrease in fresh weight, an increase in lipid peroxidation, the acceleration of electrolyte leakage, MDA accumulation, the decomposition of macromolecules, and the death (Lone et al., 2021). As with all plant growth and development processes, aging is also controlled by growth regulators (Alaey et al., 2011; Miceli et al., 2019). We found that the separate or simultaneous application of GA_3 and SA extended the vase life of *A. plumosus* versus the control irrespective of the application time by reducing bacterial load, preserving solution uptake, relative water content, chlorophyll content, and membrane integrity, and increasing the activity of antioxidant enzymes. Also, these compounds were more effective when they were applied together. The best results for these traits were obtained from the foliar application of SA_{100} + GA_{200} at the preharvest phase and the application of SA_{200} + GA_{100} at the postharvest phase. Although there were no significant differences among different rates of SA and GA_3 in improving these traits, the application of higher rates of these compounds (400 µmol) had weaker results than their 100 and 200 µmol levels. Supapvanich & Promyou (2013) state that the appropriate concentration of SA varies with plant species. In some species, its higher concentrations have adverse consequences of increasing lipid peroxidation and decreasing longevity. There are reports about the desirable effect of GA_3 on extending the vase life of *Calathea louisae* (Pinto et al., 2007), *Limonium latifolium* (Janowska et al., 2013), gladiolus (Saeed et al., 2014), and lily 'Caroline' (Zhang et al., 2022) and the positive effect of SA on the vase life of rose (Alaey et al., 2011), iris (Ramzan et al., 2018), and lily (Abbasi et al., 2019), which corroborate our findings.

Conclusions

The preservation of postharvest quality and longevity of *A. plumosus* by applying safe compounds is of high significance for its commercial value and marketability in national and international markets. SA and GA_3 are used as well-known vase compounds for improving the longevity and quality of cut flowers. However, given the different responses of species and plant organs to these extending compounds, the present research was conducted on the foliage of *A. plumosus* at the preharvest and postharvest stages. Based on the results, the application of SA and GA_3 at low rates (100 and 200 µmol) and their combined effect improved the postharvest longevity of *A. plumosus*. The most effective treatment for improving the vase life and related traits was SA_{100} + GA_{200} in the foliar application and SA_{200} + GA_{100} in the vase solution application. These two treatments are, therefore, recommended for extending the postharvest shelf life of this plant species.

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BIODIVERSITY, AUTECOLOGY AND *STATUS* OF AROMATIC AND MEDICINAL PLANTS IN GEOPARK M'GOUN (MOROCCO)

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Abstract – The M'Goun Geopark vegetation is represented by rich Mediterranean communities composed by *Quercus*, *Juniperus*, *Tetraclinis*, Aleppo pine with high species diversity, particularly medicinal and aromatic ones. Such a richness, depending on region distinct characteristics, generates an ecological diversity as result of climate variation along altitudinal and continental gradients on one hand, and lithological and orographic ones on the other. The main objective of this study is to analyze the specific richness of aromatic and medicinal plants in the Geopark M'Goun (Morocco), their autecology and their *status*. The specific richness of aromatic plants was calculated by 37 linear transects along which species were gathered and counted: 47 species among 396 total species within a 5700 Km^2 area, of which at least six cultivated, are present, confirming a good adaptation of aromatic plants to the ecological conditions of the Geopark. Canonical Correspondence Analysis (CCA) was applied to the *dataset* to verify the relationships between species and environmental factors. Climate, altitude and substrate type resulted the most important factors influencing species richness and distribution. The *status* of species was detected according to IUCN Red List showing that 42 % of these species are not yet evaluated, 3 are close to threat *status*, 2 are vulnerable, and one is endangered. Some of non-evaluated species are under threat, therefore needing of assessment, mentoring, and conservation projects.

Keywords: medicinal plants; aromatic plants; species richness; ecological diversity, biodiversity management.

Introduction

The high plant diversity of the Mediterranean Region has attracted a major attention over last few decades (Cowling et al., 1996); typical features of this Region include an unusual geographical and topographical variability, a pronounced climatic biseasonality, as demonstrated by hot and dry summer and cold and rainy-humid winter, associated with an exceptional plant and animal diversity (Scarascia-Mugnozza et al., 2000): Moreover, due to the increase of the human impact there is an urgent need to identify the greatest endangered biodiversity sectors (Medail & Quezel, 1997). Morocco, as a part of the Mediterranean Region, plays a significant role in keeping plant diversity.

Moroccan vegetation has an outstanding diversity due to several factors; namely the geographical location, which allows a diversity of climates and bioclimatic stages: arid, semi-arid, and sub-humid, favoring the colonization of particular plant assemblages, not to mention the presence of multiple natural environments, as lakes, dayas, estuaries, rivers, coastline, plains, high mountains, deserts etc. In addition, there is a topographic diversity owing to the presence of two mountain ranges: the Rif in the North, the Atlas in the center and the South with plateaus, plains, deserts and 3500 kilometers of coastline; therefore, an extremely diversified lithological and edaphic substrates exist. Thus, becoming the crossroad for these several features, Morocco has its unique diversity.

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Morocco offers a significant potential for the cultivation of medicinal and aromatic plants (MAP) (USAID, 2006); its MAPs flora is remarkable by its richness, diversity and socio-economic values (Fennane & Rejdali, 2016); for example, the exploitation of rosemary (*Rosmarinus officinalis* L.) provides approximately 81,000 JT/year, which corresponds to a value of 4,050,000.00 Moroccan Dirhams (MAD) (approximately 405 000 Euros) (Ministry of Agriculture, 2005). Moroccan wild flora with more than 4200 species is distributed in 41 ecosystems (Rhafouri et al., 2015): 600 species have aromatic or medicinal qualities (Rhafouri et al., 2015; Radi et al., 2022) and 800 have aromatic and/or medicinal interest (Zrira, 2017).

According to the High Commission for Water and Forests, only 2% of the MAPs exploited are cultivated, whereas the majority of MAPs are natural (HCEFLCD, 2018). According to the national agency for aromatic and medicinal plants, Morocco is the twelfth largest exporter of MAP in the world with a rate of 52,000 tons of plants and 5,000 tons of essential oils (ANPAM, 2020). Among 600 aromatic or medicinal species, only 80 are currently exploited (HCEFLCD, 2018). The main spontaneous MAPs encountered in the forest and collective lands are rosemary, white wormwood, thyme, laurel, wild chamomile, carob tree, oregano, lavander, mastic tree, myrtle, irguel, etc (Fennane & Rejdali, 2016). The socio-economic role of MAP is not to be denied; the export revenues generate more than 615 million MAD and offer more than 500,000 working days with a total income of 25 million MAD (ANPAM, 2020).

The main exploited MAPs in Morocco are *Thymus satureioides*, *Rosmarinus* sp., *Ceratonia siliqua*, *Artemisia* sp., *Laurus nobilis* (ELKacimi, 2020); some of the exotic Moroccan medicinal plants is Argan (*Argana spinosa* Skeels), *Artemisia herba-alba* Asso, Atlas Cedar (*Cedrus atlantica* Mannerti), *Laurus nobilis, Laurus azorica*, *Myrtus communis* (Zrira, 2017), at least 15 Moroccan endemic species are exploited as aromatic and/or medicinal plants, such as *Acacia gummifera, Argana spinosa, Cladenthus scariosus, Lavandula maeirii* and *Thymus riatarum* (Fennane & Ibn-Tattou, 1998). However, medicinal plants have received little attention from researchers in the region. Therefore, there aren't many references available (CNEARC et al, 2004). Numerous issues affect the country's aromatic and medicinal plant industry, limiting its growth. Thus, it is up to the experts to combine their efforts to structure and optimize it (ELKacimi, 2020).

In relation to data above mentioned, it is clear that aromatic and medicinal plants can help to improve the lifestyle of the local population and save natural resources. Moreover, it is necessary to rationalize the exploitation of these plants to guarantee sustainable exploitation. M'Goun Geopark is highly selected to emphasize the importance of PAMs in Morocco.

Thus, the aim of the present work is to provide a synoptic view of PAMs found by our field sampling of species, their inventory and classification and finally to take a view on the IUCN *status* of each species. Therefore, this work may help to draw the alarm on the risk that threatens the future of the Morocco region from naturalistic as well as economic point of view.

Materials And Methods

Study area

The Geopark is a part of the Azilal province, located in the center of Morocco, belonging to the Central High Atlas. The area of the Geopark, displayed in figure 1, recognized by UNESCO is 5700 Km^2 containing 15 rural municipalities, home to a 200,000 inhabitants (Association du Géoparc du M'GOUN & UNESCO, 2019). It is located some 100 Km North-East Marrakech. The Geopark has an exceptional geological history dating back to the Triassic period (250 million years ago), however, the main stages took place during the Jurassic (180 million years ago) (UNESCO, 2015) producing an outstanding geological and topographic features and geo-sites: Ouzoud geo-site, Cathedral Mesfrane-geosite, Imi nifri natural bridge, pink terrains, red clay of Azilal, etc.; it has a variety of topographical and climatic characteristics, soils are generally mountainous, and bioclimatic belts are ranged between semi-arid or sub-humid to humid (rainfall between 550 mm and 700 mm in Azilal and up to 1000 mm in the High Atlas) (Taïbi et al., 2015). The character of the climate is Mediterranean, characterized by a cold winter and a hot summer (Ionesco and Mateez, 1964), which leads to significant differences in temperature ranged between negative values in the winter and about 40 °C in the summer. The dominant vegetation is represented by Q*uercus ilex* L. forest spread between 1100 m and 2400 m a.s.l. in high mountains. *Juniperus oxycedrus* L. forms colonies in reduced forests at 1250 m persisting until 2100 m. *Buxus sempervirens* L. and *Buxus balearica* Lam. are the most remarkable species associated with *Quercus ilex* oak groves. The thorny xerophytes represent a Habitat where the most common species are *Cytisus balansae* (Boiss.) Ball., *Alyssum spinosum* L. and *Bupleurum spinosum* Guan., colonizing the extreme elevations (from 1800 to about 3000 m), where temperatures are exceptionally cold during the winter, accompanied by winds and significant snowy rainfall. Pine forests (*Pinus halepensis* Mill.) exist in the regions of Tillouguit near the Cathedral Mesfrane cliffs,

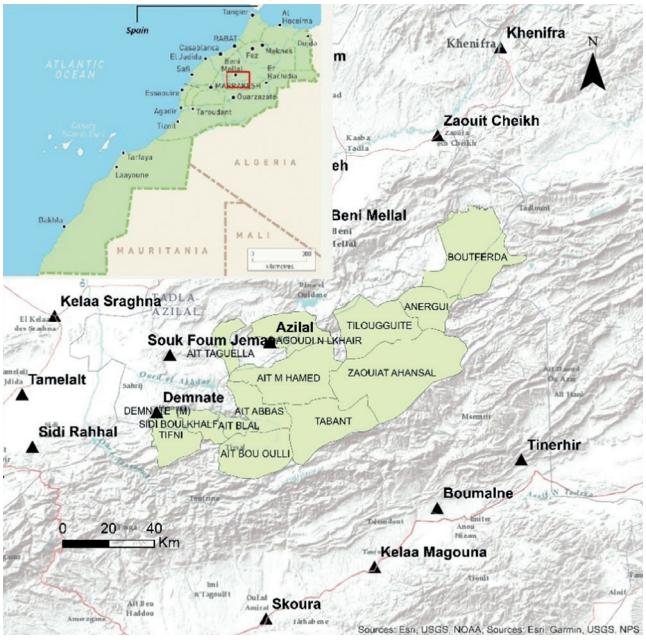

Figure 1. Study area location.

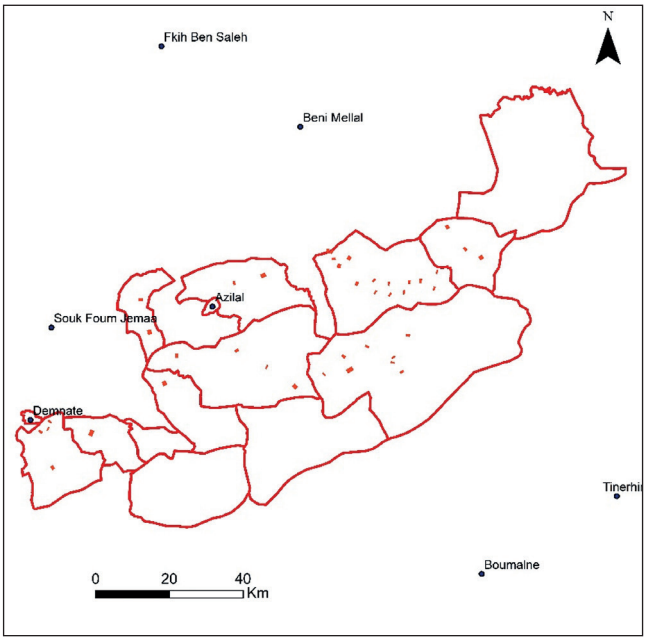

Figure 2. The transects map.

Ait Abbas, ZauitAhensal and Demnat where altitudes vary between 1200 and 1700-1800 m. a.s.l.

This area was chosen because it's a protected area, containing the most important Moroccan habitats, forming a home for an outstanding biodiversity, especially in the Atlas Central, a crossroad of the most important Moroccan ecosystems and environments, Middle Atlas in the West, High Atlas in the East, Tadla plain in the North and the desertic arid domain in the South.

Sampling Methods

The methods of sampling were developed to permit accurate estimations based on the goals pursued, like the extent of the vegetation, its characteristics, and the resources available (Glèlè, 2016). Among the numerous sampling techniques the sampling method adopted in this study is the random sampling, and stratified sampling; it means that instead of sample all the Geopark habitats sampling process will occur once in each habitat, which requires a forest cover relatively homogeneous (Bouxin, 2011); its use is justified by the redundancy of the same plant communities all over the area at large scale. In the ground the different size of transects were managed to sample the species and track their presence, abundance and the soil type.

Method of transects, more suitable with the study area feature, especially in this mountainous topography, was used. In total we realized 37 transects with different size comprised between 200 m and 500 m. Transects were linear, along which species were gathered and numbered. Inside each transect we created 5 plots, each of about 100 m^2 depending on the environmental characteristics (slope, river presence, rockiness etc.) (Figure 2). In each plot data were collected: coordinates, species presence-absence, soil type, etc. Sampling was carried out within the different Geopark's habitats, especially Aleppo pine (*Pinus halepensis* L.) forests, Holm oak (*Quercus ilex* L.), cushions of xerophytes, red juniper (*Juniperus phoenicea* L.) associated with *Tetraclinis.* In the field work, the species were recognized, sampled and classified. This phase is the most crucial being the basis of the investigation, with the identification and localization of the species, using the practical flora of Morocco (Fennane & Ibn-Tattou, 1998, 1999), the Synonymic Index of the flora of North Africa (Dobignard & Chatellain, 2010, 2011a, 2011b, 2013). Classification adopted was the APG III and APG IV(The angiosperm phylogeny group, 2009, 2016).

Climate

The climate parameters (precipitation, mean, minimum and maximum temperatures) and GIS (Geographical Information System) data were gathered. After, maps of topography, precipitation, temperatures, climate and bioclimate, geology and soils were realized. The results were then combined by projecting plants coordinates plots on these maps. Determining the different locations of each species and its extent allow the determination of its autecology by restoring the climate features (Precipitations, Tmin and Tmax), bioclimate, lithological, and topography using the superposition of layers under ArcMap software, and the distribution of species.

Statistical Analysis

The DEM (Digital Elevation Model) of the study area is used to determine the altitudes and elevations from USGS site (Usgs, 2022), the geological map is used to summarize the main substrates (Commission de Topographie marocain, 1971), and the site Worldclim serves as the climate data source (Fick & Hijmans, 2017).

The bioclimatic map is built using the Pluviometric Quotient (Q2) of Emberger (1930) for the Mediterranean climate zone: Q2= (P*2000)/(T-t) (T+t)

Where P represents the total annual precipitation, T is mean maximum temperatures for the warmest month and t is mean minimum temperatures for the coldest month (Marres, 1930). The Q2 permits the characterization of the different bioclimatic zones, and t the minimal temperature of the coldest month allows the definition of the climatic variants (Daget, 1977; Quézel & Barbero, 1982). The Mediterranean vegetation strata (see Figure 11: thermo-Mediterranean, meso-Mediterranean, supra-Mediterranean, etc.) are related to the vertical plant distribution, build basing on the correspondence created by Emberger, Quezel and Berbero (Achhal et al., 1979).

In order to track the vegetation status through the region, we will use the IUCN Red List Criteria (International Union for the Conservation of Nature), the package Red List "taxize" in R allows to determine the species categories (Chamberlain et al., 2022). The IUCN categories and criteria classify species into nine groups: Extents (EX), Extent in the wild (EW), Critically endangered(ED), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), Data Deficient (DD), and Not evaluated (NT) (IUCN, 2022).

CCA (Canonical Correspondence Analysis) is used to study statistically the dependency of the medicinal and aromatic

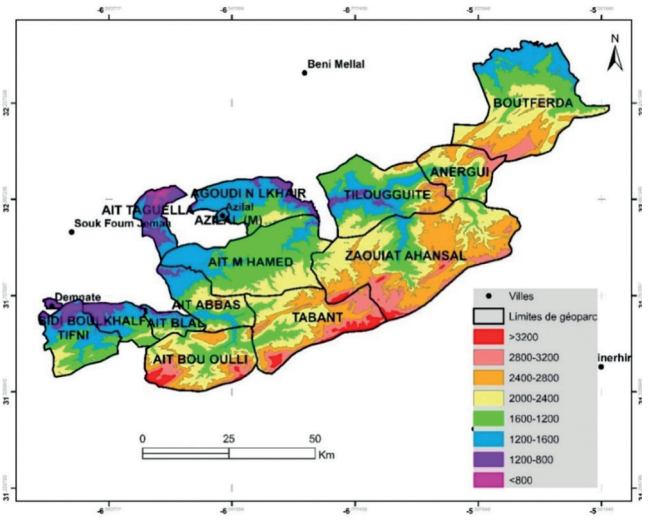

Figure 3. Geopark M'Goun altitudes map.

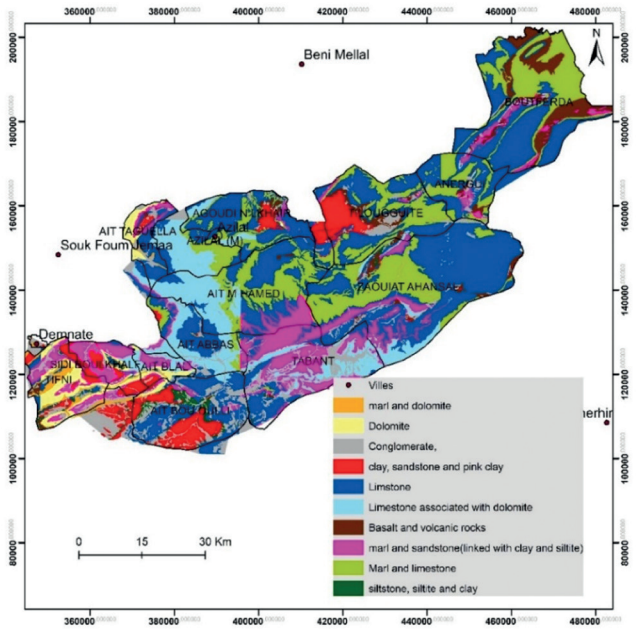

Figure 4. Different substrate types of the Geopark M'Goun.

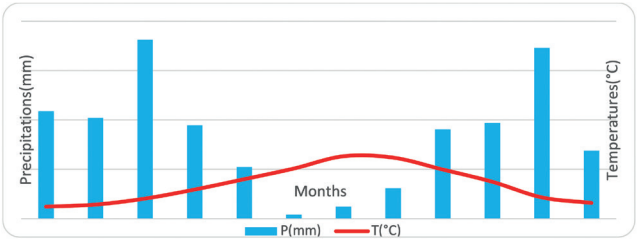

Figure 5. Climogram of Demnat, 900m.

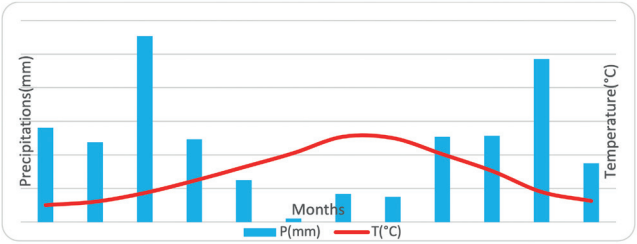

Figure 6. Climogram of ZaouitAhensal, 2000m.

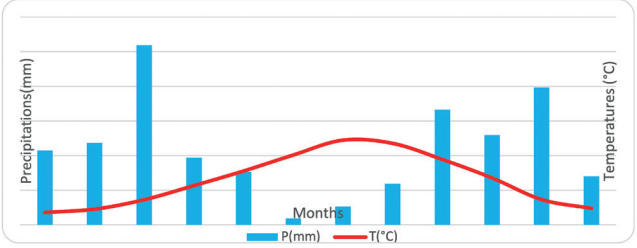

Figure 7. Climogram of AitBougmmaz (Tabant, 1866 m).

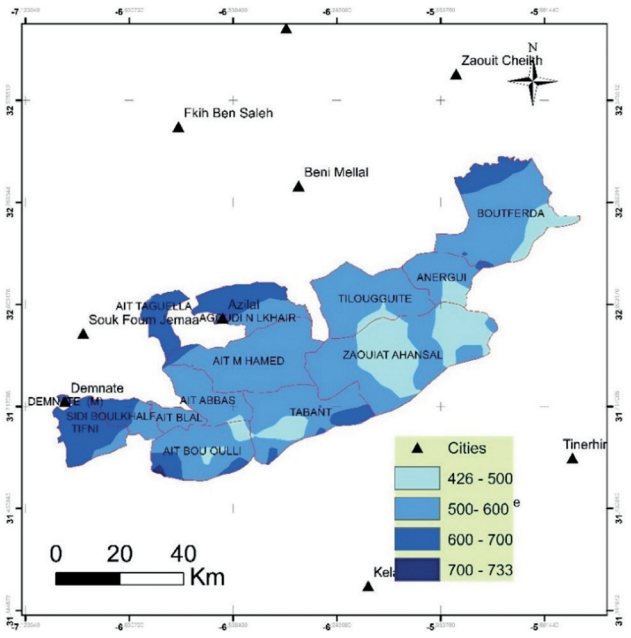

Figure 8. Precipitation map of the study area. Precipitation varies widely throughout the study area, ranging from 400 mm to 730 mm.

plants richness on the environmental factors. Since its debut in 1986 (Pillsbury & Miller, 2008), CCA has become one of the most popular multivariate methods in community ecology (Xia, 2020). This technic represents a multivariate procedure to elucidate the relationship between species and their environment (Ter Braak and Verdonschot, 1995), assuming a reasonable unimodal response curve of *taxa* to environmental variables (Xia, 2020). CCA is used when species are directly related to measured environmental factors and to explain the species distribution in the context of environmental data (Cao et al., 2011). The Past software is used for running CCA.

Results

Topography and substrates

The altitudes oscillate between 539 m and 3695 m a.s.l.. The average altitude is 1588 m. Moving southward, the topography becomes more pronounced (Figure 3).

The Azilal region's territory is a complicated area with significant substrate complexity, exposing a variety of magmatic rocks, red siltites, evaporites, and basalts underlined by limestone formations, marl, and clayey-sandstone. In general, the regions of Demnate, Azilal, and AitBoulli are characterized by detrital formations and associated with red clays and sandstones as substratum. In addition, the majority of the Geopark area is formed by carbonate formations: limestone, dolomite, or both combined (Figure 4).

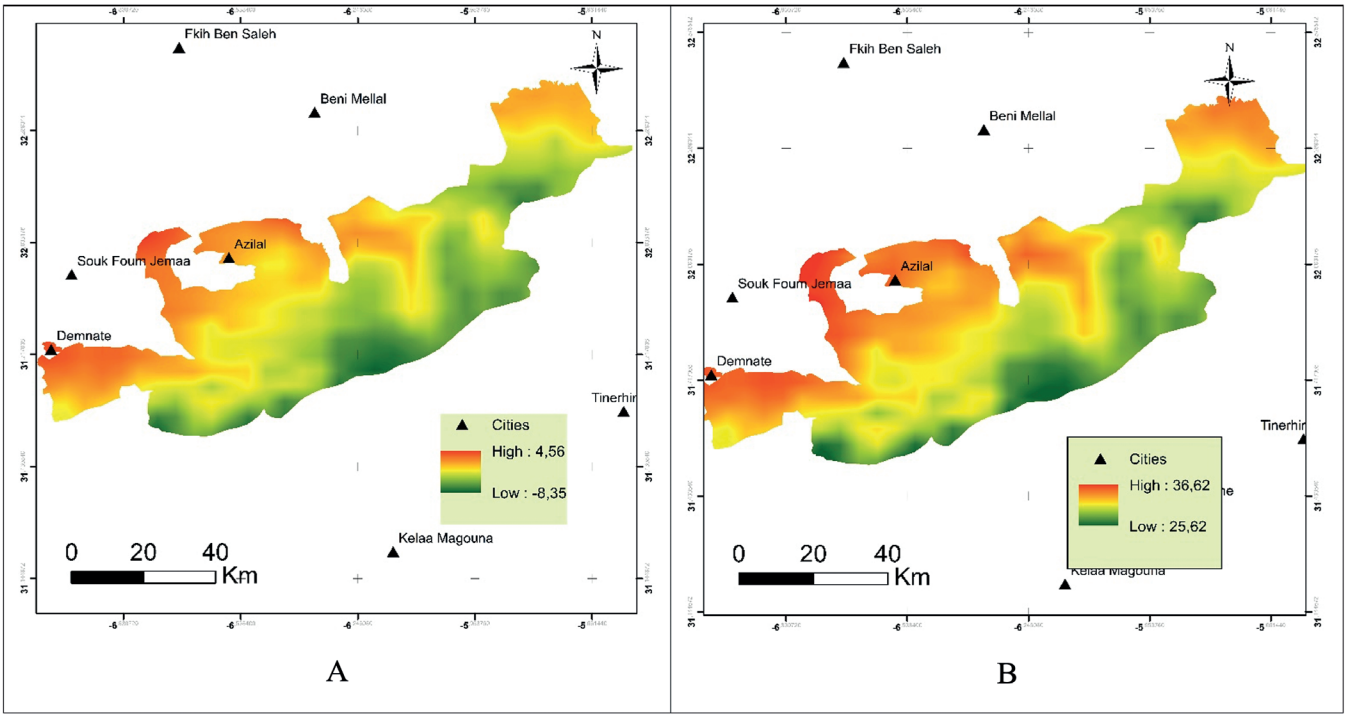

Figure 9. Temperature fluctuation in the study area. A: minimum temperature variation and B: maximum temperature variation.

Climate data

The climate of the study area is characterized by a hot and dry summer and a wet and cold winter, which demonstrates its Mediterranean character (Figures 5, 6, 7).
The temperatures vary considerably inside the geoparc M'Goun; the minimum temperatures oscillate between -8.35 and 4.56 °C, concerning the maximum temperatures, the enregistered values vary from 25.62 to 36.62 °C (Figure 9 A, B).

Bioclimate Variables

The region exposes two bioclimate types: semi-arid and subhumid; the semi-arid has a cold, fresh, and temperate winter in the low elevations and a very cold winter at the top of the mountains; however, the sub-humid in the rest of the Geopark has three winter varieties: cold, very cold, and fresh (Figures 9, 10). The region contains five bioclimatic zones: Oro-Mediterranean, mountain Mediterranean, supra-Mediterranean, meso-Mediterranean and thermo-Mediterranean (Figure 11).

Medicinal and aromatic plants

A wide variety of habitats was found in the M'Goun Geopark region, including oak groves, *Juniperus* woodlands, *Tetraclinis* matorral, pine forests, lawns, and thorny xerophytes formations. These habitats are extremely abundant in fragrant and therapeutic species: on a total of 396 species, 47 were classified as aromatic and medicinal plants, representing almost 12%.

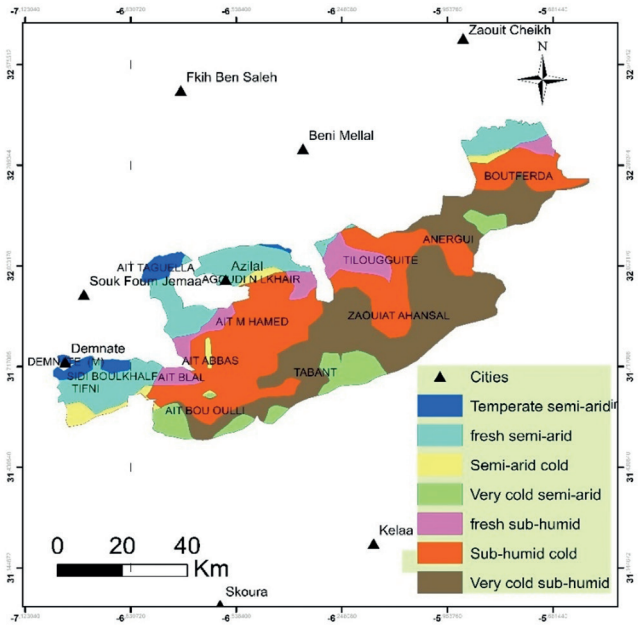

Figure 10. Bioclimatic map derived by Pluviometric quotient of Emberger.

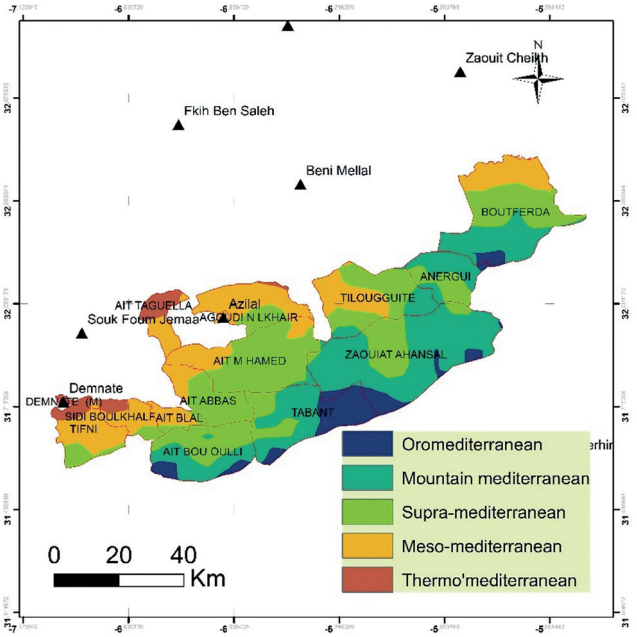

Figure 11. Map of vegetation bioclimatic zones.

Species are reported below in Table 1:

Families	Species
Lamiaceae	*Thymus satureioides* L. *Rosmarinus officinalis* L.* *Mentha pulegium* L. *Mentha spicata* L.* *Marrubium vulgare* L. *Thymus algeriensis* Boiss. &Reut *Thymus zygis* L. Mentha rotundifolia L. Salvia verbenaca L. *Verbena officinalis* L. Salvia officinalis L.* Ballota hirsula L. Thymus pallidus Coss. *Ziziphora hispanica* L. *Micromeria hochreutineri* Briq. *Ajuga iva* L.
Lauraceae	*Laurus azorica* L.
Aceraceae	*Chamaerops humilis* L.
Erecacea	*Arbutus unedo* L
Compositae	*Lavandula multifida* L. *Globularia alypum* L. Artemisia *herba-alba* L. *Pseudognaphalium luteoalbum L.* Ormenis scariosa *Ball.* *Anacyclus pyrethrum* L. *Cladanthus arabicus* (L.) Coss Globularia arabica L.
Myrtaceae	*Myrtus communis* L.*

Cupressaceae	*Juniperus oxycedrus* L. *Juniperus phoenicea* L. *Juniperus thurifera* L Tetraclinis articulata (Vahl) Mast.
Anacardiaceae	*Pistacia lentiscus* L.
Rosaceae	*Crataegus azarolus* L. *Rosa canina* L.
Fabaceae	*Ceratonia siliqua* L.
Iridaceae	*Crocus sativus* L.*
Euphorbiaceae	*Euphorbia resinifera* L.
Caryophyllaceae	*Silene vulgaris* Gracke
Oleaceae	*Olea europaea* var. *europaea sylvestris* L.
Rhamnaceae	*Ziziphus lotus* L.
Amaranthaceae	*Dysphania ambrosioides* L*.
Géraniaceae	*Pelargonium graveolens* L*.
Cistaceae	*Cistus albidus* L. *Cistus creticus* L. *Cistus lauriflorus* L.
Capparaceae	*Capparis spinosa* L.

*Cultivated species.

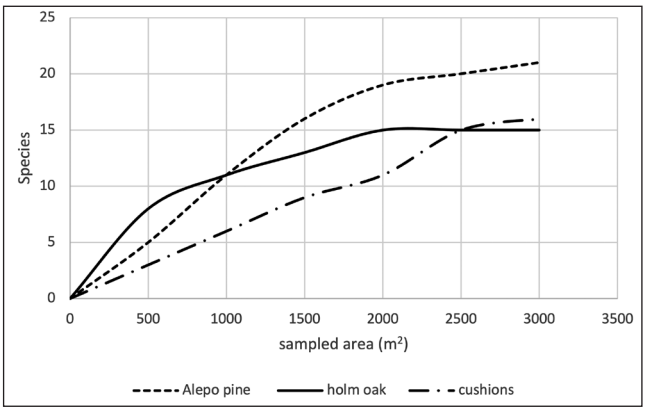

Figure 12. Species-area plot.

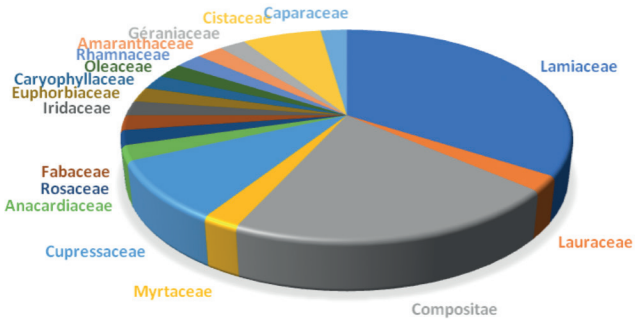

Figure 13. Medicinal and aromatic plants distributed by family**.**

Three distinct habitats are used to build the area-species relationship; Aleppo pine, Holm oak and xerophyte cushion. The curves show a variability of the species richness in each habitat type: Aleppo pine forests (*Pinus halepensis* L.) present the highest species richness, followed by cushions vegetation and finally holm oak forests (*Quercus ilex* L.) (Figure 12).

Within the M'Goun UNESCO Geopark, there are roughly **47** species of aromatic and medicinal plants, at least 6 of which are cultivated, namely *Rosmarinus officinalis* L., *Verbena officinalis* L., *Myrtus communis* L., *Pelargonium graveolens* L., *Salvia officinalis* L. and *Mentha spicata* L. *Lamiaceae* family shows the highest species number (16), followed by *Asteraceae* (9), *Cupressaceae* (4), *Cistaceae* (3), *Rosaceae* (2) and other families have one species each (Figure 13).

Canonical Correspondence Analysis (CCA)

In the CCA diagram the environmental variables are represented by arrows, species by spots: variability of the species distribution in the study area is also showed (Figure 14).

CCA1-horizontal axis represents 40.25 % of variance (p=0.006), CCA2-vertical axis 29.83% (p=0.001), CCA1 and CCA2 represent an accumulation variance of 70.08%.

CCA planes show the environmental factors correlated to species pattern. The most of species are distributed along the axis 1 which in fact explains the higher variance value. CCA1 is positively correlated with climatic variables (Tmin, Tmax, Precipitation) and with silicate substrate type; species distributed in this upper right sector of the diagram are *Cladanthus arabicus, Pistacia lentiscus, Cistus albidus, Ceratonia siliqua, Olea oleaster* etc. (see Figure 14); axis1 is negatively correlated with altitude and carbonate substrate type; species distributed in this upper left sector are *Ormenis scariosa, Juniperus thrurifera, Crategus azarolus* etc.(see Figure 14). CCA2 doesn't show significant correlations between environmental factors and species pattern.

The most of species present in the Geopark area are herbaceous aromatic and medicinal plants (Table 2). The lawns, in particular, host herbaceous species, among which different species of *Thymus* (*Thymus pallidus* Coss., *Thymus algeriensis* Boiss. & Reut., *Thymus zygis* L.), developing every year during the spring and summer, then disintegrating at the end of the year to survive during the cold season as roots. Also *Scorsonera* sp. and many *Poaceae* species colonize this habitat.

Red juniper (*Juniperus phoenicea* L.) occurred under the holm oak distribution area and may reach 1820 m a.s.l.. The

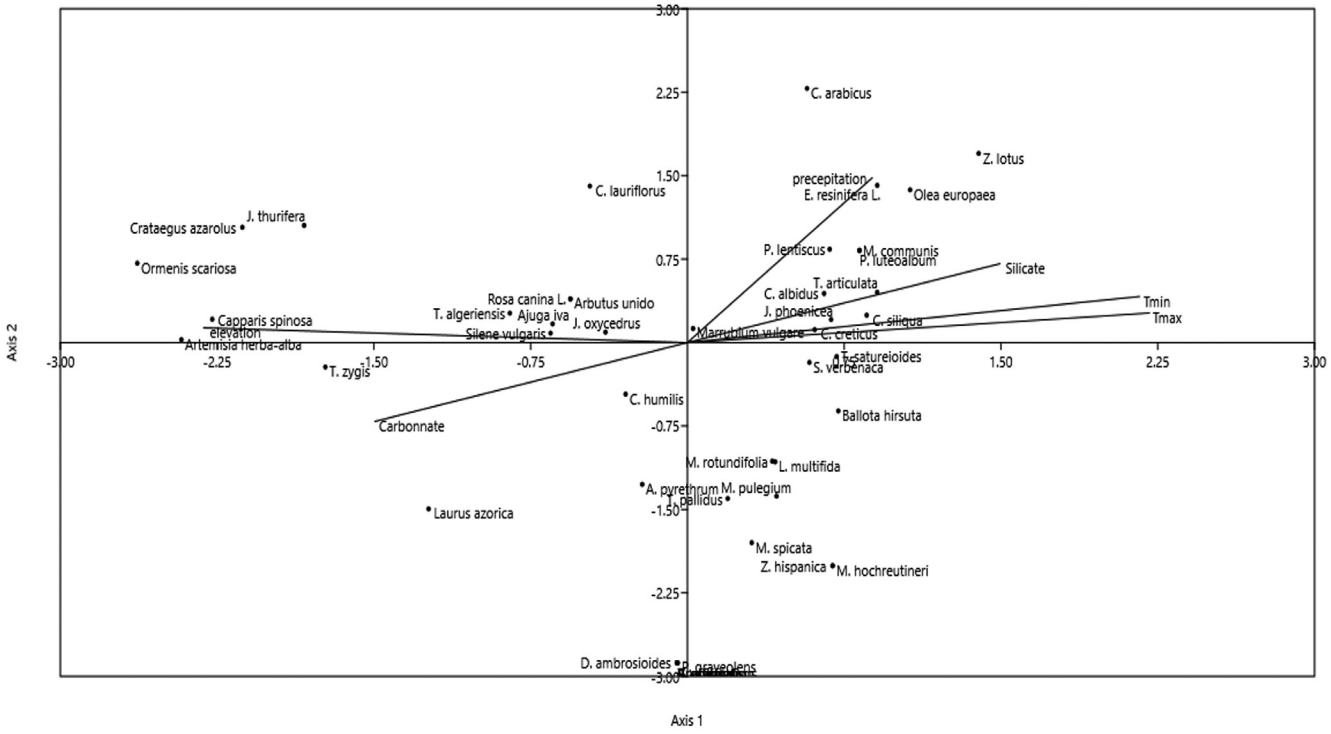

Figure 14. Relationship between species and environmental variables in the CCA plane.

Table 2. Aromatic and medicinal plants Autecology.

Species	Associated vegetation	Altitudes	Type of substrate	Climatic variants	Bioclimatic zone	Precepitaton (mm)
***Juniperus phoenicea* L.** Berber name: Kizou Arabic name: Araar	Forests and matorrals where it is the most remarkable species	reaches 1800 m	All types, essentially calcareous soils	Temperate Fresh	*Semi-arid*	500-700
Tetraclinis articulata L Berber name: Aarar Arabic name: Laaràra	Forests and matorrals; co-existes with J. *phoenicea*	Reaches 1500 m	Clay and limestone soils	Temperate Fresh	*Semi-arid*	400-600
***Juniperus thurifera* L** Berber name: Tawalt Arabic name: Laarar lfawwah	Deteriorated woodlands among the cushion of thorny xerophytes	From 1800 m	Limestone	Cold Very cold	Sub-humid	400-500
***Ceratonia siliqua* L** Berber name: Tikit, Tichit Nom Arabe: Lkherroub	Associated with juniper, oak, and thuja trees	Reaches 700m	Clays and limestones	Temperate Fresh	Semi-arid	500-700
***Olea europaea* var. *europaea sylvestris* L** Berber name: Azemmour Arabic name: Zittounberri	Associated with carob tree, thuja, juniper, mastic tree…	Presents at 1200 m	clays l, rocky cliffs and soils with calcareous mother rock.	Temperate Fresh	Semi-arid	400-700
***Juniperus oxycedrus* L.** Berber name: Tikki	It appears on degraded oak groves	From 1080m, abundant at 1250m and persists to 2100m	Limestone and clay	Cold Fresh	Sub-humid	500-600
***Laurus azorica* L.** Berber name: Taslt Arabic name: Asat Sidna Moussa	Rocky cliffs where access is extremely difficult	From 1650 m	Limestone and dolomite	Cold	Sub-humid	500-600

***Arbutus unedo* L.** Berber name: asasnou Arabic name: sasnou	Coexists with holm oak in the fresh zones	1570 m	Limestone	Fresh	Semi-arid	About 600
***Pistacia lentiscus* L.** Berber name:Tidit Nom Arabe : Drou	red juniper, thuja formations and the presence of holm oak	reaches 1600 m	Limestone and clay	Fresh	Sub-humid	400-600
***Crataegus azarolus* L.** Berber name: Admam Arabic name: Zaarour	holm oak forests and clearings	From 1700m	Limestone	Cold Fresh	Semi-arid	500-600
***Ziziphus lotus* L.** Berber name: Azeggour Arabic name: Sedra	In the hottest and most arid regions of the Geopark	Sampled up to 1300 m	Clay	Temperate Fresh	Semi-arid	600-700
***Rosa canina* L.** Berber name: Taghfert	Moist and well-drained habitat	Reaches 1600 m	Clays, dolomite and calcareous	Fresh	Sub-humid	700
***Capparis spinosa* L** Arabaic and berber name: Lkbbar	Cliffs and rocky area	1400 m to 2000m	Carbonates	cold	subhumid	400-600
***Euphorbia resinifera* L** Berber name: Tikiwt, Tichiwt Arabic name: Zeggoum	Colonizes the sunny slopes and cliffs, where co-exisst with carob trees and thuja, holm oak etc.	From 700 m to 1950 m in sheltered zone and warm areas	Limestone	Temperate Fresh Cold	Semi-arid	500-600
***Chamaerops humils* L.** *Berber name:Tgzdemt* *Arabic name:Doum*	Exposed slopes, degraded forests, and cliffs	From 1200 to 2050 m	Limestone and clays	Fresh Cold Temperate	Semi-arid Sub-humid	400-700
***Globularia alypum* L.**	Formations of thuja and red juniper	1800 meters in the Zaouit Ahensal region	Clay soils	Cold	subhumid	400-600
***Globularia arabica* L.**	Rocky and cliffy areas with less vegetation.	From 1100 m	Calcicole	Fresh	Semi-arid	400-600
***Cistus albidus* L.** Berber name: Irguel	Mostly inhabits holm oak forests	reach 1400 m.	calcareous, less prevalent on silicate soils	Fresh	Sub-humid	400-500
***Cistus creticus* L.** Berber name: Irguel	associated with *Cistus albidus* sharing similar habits	sampled until a height of 1600 meters.	Limestone	Fresh	Sub-humid	400-500
***Cistus lauriflorus* L.** Nom berbère : Irguel	Mountains and sunny slopes	Reaches 1500m	All types of soil	Fresh Cold	Sub-humid	500-600
***Thymus satureioides* L** Berber name: Azouknni Arabic name: Zaitra	Aleppo pine, holm oak, red juniper, on open areas, or in matorrals	Up to 2100 meters in ZaouitAhensal. And 1500m in Demnat	Limestone, clay and rocky soils	Fresh	Sub-humid and semi-arid	500-700
***Thymus algeriensis* L** Berber name: Tazouknit Arabic name: Zaitra	either in open regions or places with less extensive oak cover	1800 m, discovered at Tillouguit	Limestone	Fresh Cold	Sub-humid and semi_ aride	500-600
***Thymus zygis* L** Berber name: Tazouknit Arabic name: Zaitra	either in open regions or places with less heavy oak cover	sampled at Tillouguit at an elevation of 1700 m	Limestone	Cold	Sub-humid	400-600
***Thymus pallidus* Coss.** Berber name: Azouknni Arabic name: Zaitra	Clearings, rocky regions, and uncultivated fields	From about 1600 m to 2500 m	Limestone	Cold	Sub-humid	500-600

***Marrubium vulgare* L.** Berber name: Merrouyn Arabic name:Merroyt	Oak woodlands and their clearings	Sampled between 1100m and 1900m	Limestone	Cold Fresh	Sub-humideznd semi arid	500-600
Lavandula multifida L. Berber name: lkhzama Arabic name: Lkhzama	Open area and wadis	About 1800	Siliceousclay	Fresh	Subhumid	500-600
Artemisia *herba-alba* L Berber name: Tafsit, chiba. Arabic name: Chih	Juniper matorrals and clearings	Abundant at altitudes of 1800-1900 m	Limestone and dolomite	Cold Very cold	Sub-humid	500-600
Ormenis scariosa Ball. Location Berber name: Idzghi	Thorny xerophyte habitats	emerges at 1900 m common at 2500 m.	Limestone	Cold Very cold	Sub-humid	500-600
***Mentha pulegium* L.** Berber name: flio Arabic name:Flio	Wetlands (riverbanks and springs)	between 1200 and 1700 m	All soil types	Fresh	Sub-humid and semi-arid	hydrophile
***Micromeria hochreutineri* Briq.**	Degraded holm oak forests and open zones	1800 m	Limestone	Cold	Sub-humid	400-600
***Cladanthus arabicus* L.**	Shape of seasonal pastures during spring	Low altitudes in the region of Demnate	Clay soils	Temperate	Semi-arid	500-600
Mentha rotundifolia L Berber name: Timijja Arabic name: Mrsita	Wetlands (rivers and springs)	abundant at 1600 meters and can reach 2000 meters	All soil types, abundant in carbonates.	Cold Fresh	subhumid	hygrophyte
***Anacyclus pyrethrum* L.** Berber name: Aguendis Arabic name: Oud al attas	Grows naturally on clearings or uncultivated parts of fields	From 1000 to 2500 m	Limestone	Cold Fresh	Sub-humid	500-600
Silene vulgaris Berber name: Taghighacht Arabic name: Taghcht	Holm oak in clearing and fields	Wellabundant at 1800 m	Limestone	Cold	Sub-humid	500-600
***Ziziphora hispanica*L.** Berber name:Taflayout	Open areas, degraded matorrals or forests	1500 to 1700m	Limestone	Cold	Sub-humid	500-600
Ajuga iva L. Berber name:Touftlba	Rocks and rocky soil	Appears from 1800 m	Limestone	Fresh Cold	Sub-humid	500-600
Ballota hirsula L.	Inhabit the red clay and rocky soils	Reaches 1600m	clay	Fresh	Semi-arid	500-600
Salvia verbenaca L	Widespread, especially colonizes open areas	From 1200m to about 2000 m	Various soil types	Fresh and cold	Semi-arid and sub-humid	400-700
Pseudognaphalium luteoalbum* *L	Rocky and sandy soils	Sampled in 1600m	Limestone	Cold	Sub-humid	500-600
***Mentha spicata* L** Berber name : Naȧnaȧ Arabic name Naȧnaȧ	Cultivated	All elevations	All soil types	fresh and cold	Semi-arid and sub-humid	Irrigated
***Verbena officinalis* L**	Cultivated	Thrives in low elevations about 1200m	All soils types	Fresh	Semi-humid	Irrigated
Salvia officinalis L Arabic and berber name:Lwiza	Cultivated	1500 m	Various soil types	Fresh	Semi-humid and semi-arid	Irrigated

***Crocus sativus* L** Arabic and berber name: Zaàfran	Cultivated	Low altitudes	Sandy and loamy soils	Fresh	Semi-arid	Irrigated
***Dysphania ambrosioides* L** Arabic and berber name: Mkhinza	Cultivated and thrives next polluted steams	About 1300 and 1500	Organically rich soils	Cold and fresh	Semi-humid	Irrigated and humid areas
***Pelargonium graveolens* L** Arabic name *: àtarcha*	Cultivated	About 1400	Sandy and loamy soils	Fresh	Semi-humid	Irrigated
***Rosmarinus* officinalis L** Beber name: Azir Arabic name: Iklil ljabal	Cultivated and natural in the poor rocky open areas	All altitudes lowlands and uplands	Sand and clay soils	Fresh and cold	Semi-arid and humid	400- 500
***Myrtus communis* L** Arabic name: Rihan	Cultivated	Low altitudes	Various soil types	Fresh	Semi-arid	Irrigated

Berberian thuja (*Tetraclinis articulata* (Vahl) Mast.) appears at low elevations and reaches 1500 m a.s.l.; it forms forests and matorrals where it is remarkable the presence of species at low altitudes; then *Cistus* spp. leave the area gradually to the red juniper. *Globularia* and *Lavandula* species are widely distributed in these woodlands (mainly *Glubularia alypum* L. and *Lavandula multifida* L.).
Alyssum, Genista, Cytisus, and *Astragalus* are a few of the numerous *genera* of thorny xerophytes that can be found at high altitudes above 1800 m. *Ormenis* and *Thymus* are the relevant aromatic plants at this level, *Ormenis scariosa* emerges at 1900 m in the Zaouit Ahensal region, where reaches the highest abundance values at 2500 m, forming the cushion plants habitat occurring in particular places, presenting optimal environment condition such the soil deepness, protection from the wind, with a greatest abundance. Additionally, there are few isolated *Juniperus thurifera* trees spread across these cushions, which create altered woodlands in the Tillouguit, Zaouit, Ahensal, and Anergui regions.

Discussion

Species richness

Results demonstrated that species richness detected in the study area is generally high. Three habitats were selected to test the trends of the relation species/area (MacArthur & Wilson, 1967): *Pinus halepensis* forests and cushion xerophytes formations showed coherent patterns, while in *Quercus ilex* forests, when area size exceeds 2000 mq, species number no longer increases. Evidently, in a forest keeping homeostatic capacity in balance with climate and soil, species number has a threshold in correspondence of a certain area extension. On the contrary, in more open vegetation like *Pinus halepensis* forest and cushion xerophytes formations, species access is more influenced by the area size.

Aromatic/Medicinal plants

As regards aromatic and medicinal species, it is remarkable that the highest species richness was found in the *Lamiaceae* family (Figure 13), supporting a study carried out in Greece (Cheminal et al., 2020) where authors proved that this *taxon* is of great importance for its chemical composition and properties. So, also in Morocco *Lamiaceae* species can improve ecosystem services increasing the cultivation, *f.i*., of *Rosmarinus officinalis, Salvia officinalis. Mentha spicata*.

Relationship between species and environmental factors

In order to evaluate which environmental factors more influence richness and distribution of aromatic/medicinal species, CCA results demonstrated that Temperature, Precipitation, Altitude and substrate type were the most significant. Axes 1 and 2, representing a high value of accumulation variance (70.08%), explain the species distribution in the CCA diagram (Figure 14). Axis 1 was positively correlated with climatic variables (Tmin, Tmax, Precipitation) and with silicate substrate type; negatively with carbonate soil types (limestone, dolomite) and altitude. Species like *Cladanthus arabicus, Pistacia lentiscus, Cistus albidus, Ceratonia siliqua, Olea oleaster* are dominant on silicate soils, sunny slopes, forest clearings, hot shrublands and matorral.
The species group distributed on the left CCA sector is well adapted to carbonate soils and important elevations: *Ormenis scariosa*, *Artemisia herba-alba*, *Crataegus azarolus*, *Juniperus thurifera*, *Capparis spinosa* and *Thymus zygis,* belonging to xerophytes habitat. A second group is

Table 3. IUCN species categories.

Species	IUCN Categories
Rosmarinus officinalis *Mentha pulegium* *Mentha spicata* *Thymus zygis* *Verbena officinalis* Salvia officinalis *Laurus azorica* *Globularia alypum* *Pseudognaphalium luteoalbum* *Myrtus communis* *Juniperus oxycedrus* *Juniperus thurifera* *Pistacia lentiscus* *Crataegus azarolus* *Rosa canina* *Chamaerops humilis* *Ceratonia siliqua* *Silene vulgaris* *Cladanthus arabicus* *Arbutus unedo*	LC
Marrubium vulgare *Juniperus phoenicea* *Olea europaea*	NT
Mentha rotundifolia *Anacyclus pyrethrum*	VU
Tetraclinis articulata	EN
Thymus satureioides *Thymus algeriensis* Thymus pallidus Artemisia *herba-alba* Ormenis scariosa *Crocus sativus* *Euphorbia resinifera* *Ziziphus lotus* *Dysphania ambrosioides* Ballota hirsula *Pelargonium graveolens* *Cistus albidus* *Cistus creticus* *Cistus lauriflorus* *Capparis spinosa* Globularia arabica Salvia verbenaca *Lavandula multifida* *Ziziphora hispanica* *Micromeria hochreutineri* *Ajuga iva*	NE

represented by species moderately influenced by altitude: *Laurus azorica*, *Silene vulgaris*, *Thymus algeriensis*, *Rosa canina, Arbutus unedo*, *Ajuga iva*, *Cistus laurifolius*, *Juniperus oxycedrus*, *Anactylis perethrum*, *Chamaerops humilis*; the most of these species are spread in holm oak habitat. In high altitudes *Laurus azorica* is reduced in solitary individuals, suffering its extensive exploitation that has confined its range to rocky cliff.

Species sensible to elevation like *Ceratonia siliqua*, *Tetraclinis articulata*, *Pistacia lentiscus*, *Phoenicea articulata, Thymus satureioides*, *Cistus albidus*, *Cistus creticus* inhabit especially the Aleppo pine, red juniper or *Tetraclinis* habitats. Other species don't tolerate high altitudes like *Euphorbia resinifera*, *Olea europaea* var. *europaea sylvestris* and *Ziziphus lotus,* colonizing the most arid and drought habitats in the Region.

The temperature plays an antagonist effect on species compared to elevation, since the temperature obviously decreases with altitude: these two climatic variables, displayed in fact on the opposite side of CCA1 (Figure 14), more influence the PAMs in the study area.

Mentha species were distributed on right side of CCA (Figure 14), correlated with silicate soils; generally, in fact, they were found on soils rich in silicates (clays) rather than carbonates.

In synthesis, according to bioclimatic stages (Figure 10), CCA results (Figure 14) and species autoecology (Table 2), we can summarize as follows:

vegetation referred to oro-Mediterranean stage is mainly characterized by xerophytes. The mountain Mediterranean level is inhabited by *Juniperus thurifera* and xerophytes, among which the most dominant PAM is *Ormenis scariosa*; it emerges at 1900 m. in the Zaouit Ahensal region, where reaches the highest abundance values at 2500 m, forming the cushion plants habitat occurring in particular places that are protected from severe climatic condition, with a greatest abundance. *Alyssum, Genista, Cytisus* and *Astragalus* are a few of the numerous *genera* of thorny xerophytes that can be found at high altitudes above 1800 m. Additionally, there are few isolated *Juniperus thurifera* trees spread across these cushions, which create altered woodlands in the Tillouguit, Zaouit, Ahensal and Anergui regions.

The supra-Mediterranean stage is mainly colonized by the sclerophyll forest of oak groves associated essentially with *Buxus*. The meso-Mediterranean stage is dominated by oak groves accompanied by *Juniperus oxycedrus*, Aleppo pine forests, and the top level of *Juniperus phoenicea*; this zone is highly rich in *Thymus* species and other PAMs (Table 2).

Finally, the thermo-Mediterranean stage is characterized by a large species diversity represented by *Juniperus phoenicea, Tetraclinis articulata*, *Ceratonia siliqua*, *Olea oleaster*, *Pistacia lentiscus, Ziziphus lotus* etc. (see again Table 2).

The distinctive MAP of the Ait Bougmaz region is Artemisia (*Artemisia herba-alba* L.); it grows primarily on limestone substrates on the borders of wadis and mountain slopes, as well in uncultivated areas with less grazing. The pyrethrum (*Anacyclus pyrethrum* (L.) Link), which is practically extinct, is found on limestone grounds above 1800 meters. However, trials of its cultivation on these grounds were successful. The *Capparis spinosa* L. species also inhabits the cool cliff areas.

In the Tillouguit region and in the center of the Geopark, the dwarf palm (*Chamaerops humilis* L.) colonizes the sunny slopes in the form of circular colonies linked to holm oak degraded areas. In the region of Ouzoud, Demnate and Azilal, *Euphorbia resinifera* L. thrives on calcareous exposed slopes and cliffs, reaching 1900 m in the warm and protected zones. *Zyziphus lotus* is also confined to the hottest and most arid sites. Moving southward, in the Anergui and ZaouitAhensal regions, the slopes are characterized by cushions of *Genista* species.

JUCN Evaluation

The study showed that most of the species (44%) are not evaluated in the IUCN Red List (Table 3); this may affect negatively the conservation plans, however, the 42% of species are not affected by the exploitation or is slightly exploited. In addition, three aromatic and medicinal species in the region are near threatened (*Marrubium vulgare, Juniperus phoenicea, and Olea europaea)*, 2 are vulnerable (*Mentha rotundifolia*, *Anacyclus pyrethrum)* and one is endangered (*Tetraclinis articulata)*.

The overexploited species in the region are *Thymus satureioides* L, *Juniperus oxycedrus* L, *Artemisia herba-alba* L, *Marrubium vulgare* L, *Anacyclus pyrethrum* L.; therefore, the control of their harvest is required. Some of these species are not indexed in IUCN Red List, like *Thymus satureioides,* a threatened species (Rankou et al., 2020). Other plants, particularly the trees with poor renewal rates, as thurifer juniper, suffer of intensive deterioration for therapeutic purposes and tar manufacturing; they are threatened in relation to overexploitation.

The spontaneous growth of several natural species demonstrates their optimal adaptation to the Geopark's ecological conditions. Initiatives for the production and marketing of one of the most valuable fragrant plants have recently been established in the Geopark and the Azilal region. Saffron (*Crocus sativus* L.) has been dubbed the "Red Gold" and trials of its introduction have yielded positive results.

Conclusions

The region is home to a large specific richness in general, and aromatic and medicinal species particularly as a result of a set of environment variables and ecological diversity. The M'Goun Geopark region, Azilal in general, and other locations with comparable characteristics (the Atlas) present potentialities and chances for investment in several agricultural fields, particularly the still-young and promising field of aromatic and medicinal plants. Aside from that, individuals are adapting their lifestyles to include the use of bio products, healthy practices, and environment protection. Since the activity of the population directly depends on the forest, there are numerous threats to the natural resources in this area. Therefore, by supporting initiatives to cultivate aromatic plants, new revenue streams can be generated, easing the constant strain on natural resources, these plants should be managed rationally, support and monitoring programs should be developed.

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KINETIC STUDY OF A SINGLE AND BINARY BIOSORPTION OF CADMIUM AND LEAD ONTO THE DEAD AQUATIC PLANT *LEMNA GIBBA*. BIOSORPTION OF HEAVY METALS BY A DEAD AQUATIC PLANT

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Abstract – Cadmium and lead are recognized as toxic heavy metals even at low concentrations. Thus, their removal is required. The present paper deals with the use of a natural low-cost and environmentally friendly material as a bioadsorbent obtained from a dead aquatic plant (Lemna gibba). The biosorption of Cd and Pb individually or in combination was studied under different experimental conditions such as time effect (0-240 min), concentration of metal ion (0.1 and 1 mg/L), adsorbent dose (0.10, 0.25 and 0.50 g) to examine the operational factors impact on heavy metals removal effectiveness. The dead biomass was characterized by FTIR to provide information about the functional groups responsible for biosorption. Inductively coupled plasma atomic emission spectroscopy was employed to perform quantitative measurement of Cd and Pb (ICP-OES). For the kinetic investigation, pseudo-first order and pseudo-second order, models were used.
The experimental results demonstrated that lead and cadmium adsorption onto Lemna gibba powder occurred quickly, with equilibrium being reached in 120 minutes and 30 minutes, respectively. At 0.1, the greatest removal efficiencies were 84.01% of Pb and 93% of Cd. At 1 mg/L, 73.82%, and 88% of Pb and Cd were removed respectively. After 180 minutes, both metals were effectively eliminated (90%) from the binary system that was contaminated with 0.1 mg/L of each metal. At 1 mg/L for each element, Pb was removed 86% after 90 minutes, and Cd clearance was less (54%).

Keywords: biosorption; dead aquatic plant; cadmium, lead, binary mixture, Kinetics

Introduction

Contamination of the aquatic environment by different pollutants is a serious global problem. The continuous growth in population, the expansion of urbanization and the rapid development of industrialization led to the release of organic matters and heavy metals (Lalevic et al., 2012; Rezania et al., 2015; Hu et al., 2016; Khallaf et al., 2018; Cao et al., 2019)
Among hazardous contaminants, heavy metals are a common environmental threat (Chiban et al., 2016). The pollution of ground and surface waters with heavy metals is a widespread and a serious problem (Benhima et al., 2008). With the expansion of industry, large quantities of heavy metal–contaminated water are being discharged into the environment (Lin et al., 2020). Heavy metals considered as the most important groups of water pollutants, are toxic (Benhima et al., 2008). The main sources of metal pollution, are natural (marine phosphates, weathering including erosion, non-volcanic soil, volcanic soil and volcanic activity) (Kumar et al., 2021) and anthropogenic sources (mining and smelting of metalliferous ores, burning of fossil fuels, wastes and sewage, pesticides and fertilizers (OECD, 2003) (Torbati & Keshipour, 2020; Hemalatha et al., 2021). Every year, the aqueous environment receives an average of millions tons of heavy metals as a consequence of human activities (Hu et al., 2016; Zhang et al., 2012).

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Cadmium and lead, among the most dangerous heavy metals, are frequently used in industrial processes (Volesky, 1991; Low & Lee, 1991; Chiban et al., 2011). They have no biological function (Chojnacka, 2010) and are harmful to aquatic ecological life, living organisms and human beings, even in low dosages (Fry et al., 1992; Zhang & Shao, 2013; Sheehan et al., 2014; Khan et al., 2015; Ayaz et al., 2020; Benhima et al., 2008). These heavy metals enters the food chain through potable water and sea foods, which endangers human life (Hemalatha et al., 2021).

Cadmium is a dangerous pollutant released from metal plating, ceramics, mining, electroplating, and the waste of used nickel-cadmium batteries (Chen et al., 2015; Martins et al., 2004; Ayaz et al., 2020), sewage sludge, cement industry, fuel combustion, power stations, protective plating on steel, Polyvinyl chloride (PVC) stabilizers, phosphate fertilizers, plastics, glass as a pigment, electrode material in nickel-cadmium batteries, mining activities and zinc smelting in various alloys (Huang et al., 2017; Malyan et al., 2019; Rehman et al., 2015; Sharaff et al., 2020; Singh et al., 2018; Kumar et al., 2021).

Cadmium is carcinogen (Kim et al., 2015; Kumar et al., 2021) and mutagenic (Beyersmann & Hartwig, 2008; Kumar et al., 2021). It can cause bone damage, hypercalciuria, hypertension, lung inefficiency, liver damage, renal dysfunction and neurological disorders in humans (Bernard, 2008; Cabral-Pinto et al., 2020; Kumar et al., 2021). Cd related health risk depends on its oxidation form and entry route (inhalation, ingestion) (Genchi et al., 2020). Acute exposure to Cd inhalation causes respiratory tract injury, interstitial pneumonia, pulmonary oedema and impairment of lung function. Chronic exposure of Cd will be seen in the bone, kidneys (proteinuria, renal stones, etc.) and causes Itai-Itai disease (Rahimzadeh et al., 2017; Kumar et al., 2021). Cd ingestion also affects the cardiovascular system, gastrointestinal tract, nervous system, kidneys and liver (Kumar et al., 2021).

Lead (Pb) is the second highest priority toxic heavy metal (Mal et al., 2021). Lead is of particular interest not only for its toxicity but also, by its widespread presence in the environment (Abdel-Halim et al., 2003).

Water resources are polluted with lead through various industries including electronics industry, metal-metallurgical industry, paint industry, oil refinery and mining industry (Mackay et al., 2013; Povedano-Priego et al., 2017; Mal et al., 2021), ceramics, paint, plastics, pesticide, automobiles, cement, and steel (Awual, 2017, 2019; Giri et al., 2022). The battery industry is considered as the major cause of water pollution (Roy et al., 2021; Badawy & Naguib, 2021).

Lead poisoning can cause various diseases which threaten human organs like brain and central nervous system, bony tissues development, gastrointestinal tract, kidney and liver (Zhang et al., 2019; Zhao et al., 2020; Hou et al., 2019; Badawy & Naguib, 2021). When discharged in the environment, the concentration of lead ion increases many folds and persist for a long time in soil, ground and surface water bodies. Thus, it enters in to the biological systems and affects living organisms. Its toxicity has severe effects on photosynthesis, nitrogen metabolism and cell division in plants (Wani et al., 2015; Giri et al., 2022).

Unlike organic pollutants, metals are non-biodegradable. The hypertoxicity of these metals leads to severe ecological effects. Thus, efficient processes have to be developed to eliminate them before their release into the environment (Benhima et al., 2008).

According to the World Health Organization (WHO), the maximal admitted concentration levels for cadmium and lead are 0.005 and 0.05 mg/L, respectively (Van der Leeden et al., 1990; Benhima et al., 2008; Dongre, 2020; Mal et al., 2021).

Many techniques have been developed to treat heavy metal-polluted aqueous medium (Munter, 2013). For the elimination of cadmium and lead, a variety of physico-chemical methods exist. These methods include coagulation/flocculation, chemical precipitation, ion-exchange and adsorption (Chen et al., 2020; Wang et al., 2020; Torbati & Keshipour, 2020).

Usually, these strategies are too costly (High costs of equipment and high-operational costs) (Chen et al, 2015), and inefficient to reduce heavy metals concentration to the level required by water quality standards (Abdel-Halim et al., 2003). Moreover, they might produce a toxic waste that needs additional treatment (Saleh et al., 2020; Hu et al., 2016). New innovative technologies for water treatment are required (Chojnacka, 2010). Therefore, the research is oriented towards low cost and eco-friendly technology. Green technology as phytoremediation, has received a considerable attention (Sabreena et al., 2022) and is widely used. This environmentally friendly method has been successfully able to treat heavy metal polluted sites. Phytoremediation experiments using duckweed (*Lemna gibba*) have achieved high efficiency in assimilating large quantities of heavy metals (cadmium and lead) and nutrients (nitrate and phosphate) (Aggoun et al.,2018; Aggoun & Benmaamar, 2019). Many other experiences confirm the efficiency of *Lemna* sp. in the phytoremediation of heavy metals and different organic pollutants (Ali et al., 2016; Ekperusi et al., 2019).This phyto-process is successful but it has its limitation in heavy metal removal. The toxicity of these contaminants, can reduce duckweed biomass production or leads it to death (Satyakala & Jamil, 1992; Delgado et al., 1993; Miretzky et al., 2006).

In recent years, dried plants have been used in treatment of arsenate, nitrate, phosphate, cadmium and lead ions contaminated wastewaters (Chiban et al., 2011; Moussa et al., 2015).

The use of dead, dried aquatic plants, for metal removal as a biosorbent material has advantages. They are naturally renewable, and they process more quickly (Ighalo & Adeniyi, 2020).

In our previous works, experiments were conducted to explore the efficiency of the duckweed *Lemna gibba* in phytoremediation, for the removal of cadmium and lead in single and binary systems (Aggoun et al., 2018; Aggoun & Benmaamar 2019). The results showed an excellent uptake capacity of these toxic metals. In order to avoid the disadvantages of the phytoremediation by using live plant, in the present investigation, the adsorptive potential of the dead plant *Lemna gibba* as a low-cost natural material, was evaluated for Cd and Pb biosorption, individually and their mixtures. The effect of some factors such as contact time and adsorbent dose are also evaluated.

Materials and methods

Adsorbent plant material

At the beginning of spring, young fresh plants of the duckweed *Lemna gibba* were collected from a pond of north Algeria. The sampling site is located on Blida (36°36'50.7"N 2°49'48.0"E). These plants showed a greenish coloration indicating a good physiological state. Their selection was made due to their abundance.

The biomass was first rinsed with tap water, and then with distilled water, to obtain a clean biomass. This was then dried in the oven at 60°C to constant weight and then grounded by an electric mixer to produce a fine powder. Before starting the experiments, the ground dry powder of *L.gibba* is mechanically sieved to a suitable grain size (0.5 mm). The biomass powder was then prepared as described by Gardea-Torresdey et al. (1998). Briefly, 500 mg biomass sample was washed twice with 0.01 M HCl to remove any soluble biomolecules that might cause interference, and then cleaned with sterile distilled water. The sample was filtered and then dried at 65 °C for 48 h.

Chemicals

In this study, all chemical reagents used were analytical reagent grade (purity ≥99 %)**.** Stock solutions of cadmium (Cd) and lead (Pb) were prepared by dissolving $CdCl_2$, H_2O and Pb (NO_3), in distilled water. The required concentrations of Cd and Pb solutions, were obtained by dilution with distilled water.

The initial pH value was adjusted by using 0.1 N of hydrochloric acid (HCl) and sodium hydroxide (NaOH). pH value was set at 4.0 ±0.5.

Before use, all the laboratory glassware used for experiments was cleaned with detergent, rinsed with tap water, soaked in 10% (v/v) nitric acid (HNO_3) and rinsed with distilled water.

Sorption experiments

Biosorption experiments were carried out in a thermostatic shaker at a temperature of (22±2) °C, and the agitation speed was kept constant (250 rmp). In glass flasks, a known mass (0.5 g) of dried and powdered *Lemna gibba*, was introduced in 100 mL of solutions contaminated with 0.1 or 1 mg/L of cadmium (or lead). The same procedure is followed in the binary mixture (Cd+Pb) where the combined concentrations of 0.1 mg/L or 1 mg/L of each metal are used.

Several authors have shown that acidic pH values are suitable for metal sorption (Halaimi et al., 2014; Chen et al., 2015). Thus, all sorption experiments were carried out at pH of 4.0 ±0.5

Effect of contact time

The effect of contact time on biosorption, was performed for 0.1 and 1 mg/L of Cd (Or Pb). The combined concentrations of 0.1 mg/L or 1 mg/L of each metal were used to evaluate the simultaneous contamination sorption tests. Thus, a sample of dead biomass (0.5g) was added to 100 mL of Cd or/and Pb solutions at room temperature and pH-value 4.

The flasks were shaken at 250 rpm for various periods (30, 60, 90,120, 180 and 240 minutes). At the end of each adsorption period, the biomass and the solution, contained in each flask, were separated from the solution by filtration using 0.45 µm acetate cellulose membranes. The filtrates were analyzed to determine the final Cd and Pb concentration in the samples.

The removal efficiency and the biosorption capacity of Pb and/or Cd by the dead plants were reported by using Eq. (1) and Eq. (2) respectively:

$$\text{Removal efficiency; R \%} = \frac{(C_o - C_t)}{C_o} \times 100 \quad (1)$$

$$\text{Biosorption capacity; } q_t\,(\text{mg/g}) = \frac{(C_o - C_t)}{m} \times V \quad (2)$$

Where R% is the removal efficiency at each testing time, C_0 is the initial concentration of heavy metal (mg/L), and C_t is the concentration remaining in solution after each tested time of treatment (mg/L).

Effect of contaminant concentration

To evaluate the effect of Cd or Pb concentration on biosorption, two concentrations were tested (0.1 and 1mg/L). Thus, flasks containing 100 mL of medium and 0.5 g dead plant were contaminated with Cd and/or Pb at room temperature, shaking at 250 rpm, pH-value 4, and contact time corresponding to the determined equilibrium time.

Effect of biosorbent dose

Various weights of ground plants (0.10, 0.25, and 0.50 g) were added to flasks containing 100.0 mL of Cd and/or Pb solution (0.1, 1 mg/L or their mixtures) at room temperature, shaking at 250 rpm, pH-value 4, and contact time corresponding to the determined equilibrium time.

Heavy metals analysis

The final Cd and Pb concentrations were measured using inductively coupled plasma atomic emission spectroscopy (ICP-OES) (PerkinElmer, Optima 7300 V).

Analysis of Lemna gibba powder by Fourier transform infrared spectroscopy (FTIR)

The characteristics of the dead plants surface is probed by FTIR spectroscopy using a FTIR – 8201 PC, Shimadzu.
The ground dry powder of the duckweed, were pressed into slices with Bromide potassium (KBr). Slices were observed by FTIR before and after adsorption.

Kinetic adsorption models

Kinetic analysis was performed to give important information on the reaction's mechanism and pathway. It also provides data on the relationship between adsorption rate and the amount of pollutant adsorbed.
Adsorption kinetics provides a time-based measurement of adsorption uptake. The kinetic parameters give important information for designing and modelling adsorption processes (Pirzadeh & Ghoreyshi, 2014). Thus, biosorption data were analyzed with two kinetic models: pseudo-first order and pseudo-second order, according to Eq. (3) and Eq. (4) respectively (Elwakeel, 2010):

$$\mathrm{Log}(q_e - q_t) = \log q_e - (k_1/2.303)\, t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (4)$$

Where k_1 is the pseudo first order rate constant (L/ min), q_e and q_t (mg/g) refer to the amount of metal ions adsorbed at equilibrium and at time t, while k_2 (g/(mg. min)) is the pseudo second order rate constant of adsorption.

Results

Adsorbent characteristics

The process of adsorption is controlled by the molecular structure and the functional groups of the dried biomaterial (Saleh et al., 2020). As shown in table 1, the main functional groups of *Lemna gibba* prior to adsorption were the – OH and –NH stretching vibrations of amine and carboxylic groups, responsible for the broad peak at 3425.64. The peak observed at 2926.67 cm^{-1} is corresponding to the asymmetric stretching vibration of C–H bond (Sinharoy & Pakshirajan, 2019; Jain et al., 2015a; Li et al., 2017). The peak at 1653.83 cm^{-1} denotes amide stretching vibration of C=O group of carboxylic acid and the peak at 1420 cm^{-1} is due to the stretching vibration of C–H, whereas the peak at 1157.21 cm^{-1}can be assigned to the C–O stretching.
After Pb, Cd and Pb+Cd adsorption on the dead biomaterial most of the main peaks were shifted. The FTIR spectra of the powder dried plant loaded with Pb (0.1 mg/L), revealed peaks at 3445.44, 2920.21, 2369.76, 1647.08 and 1047.25 cm^{-1} and were 3425.64, 2922.47-2364.80, 1653.83 and 1047.27cm^{-1} at 1mg Pb/L.
The FTIR spectrum related to Cd adsorption on dried *Lemna gibba*, the peaks of the main functional groups are observed at the following wave numbers: 3396.29, 2930.86, 1649.64 and 1035.70 cm^{-1} at 0.1 mg/L and 3425.64, 2926.67, 1653.83, 1456.62 and 1054.99 at 1mg/L.
When Pb and Cd were fixed simultaneously on the biomass the principle peaks are located at 3551.43, 2926.67, 2369.99, 1544.74 and 1049.20 cm^{-1} in the mixture containing 0.1 mg/L of each metal. The peaks at 3287.25, 2933.47, 2373.18, 1651.73 and 1049.00 cm^{-1} are observed when dried *Lemna gibba* was loaded with 1mg/L each metal.

Effect of contact time

At constant pH-value (4.0±0.5) and ambient temperature (22°C), the effect of contact time on the retention of lead, cadmium and their mixtures, on dried *Lemna gibba* powder are depicted in figures 1 and 2.
The removal of Pb (Figure1a) increases significantly between 0 and 120 minutes, more slower between 120 and 180

Table 1. FTIR spectroscopy bands.

Cd or Pb concentration (mg/L)	Cd concentration (mg/L)		Pb concentration (mg/L)		Cd+Pb concentration (mg/L)	
0	0.1	1	0.1	1	0.1+0.1	1+1
3425.64 2926.67 1653.83 1420.0 1157.21	3396.29 2930.86 1649.64 - 1035.70	3425.64 2926.67 1653.83 1456.42 1054.99	3445.44 2920.21 1647.08 - 1047.21	3425.64 2922.47 1653.83 - 1047.27	3551.43 2926.67 1544.74 - 1049.20	3287.25 2933.47 1651.73 - 1049.0

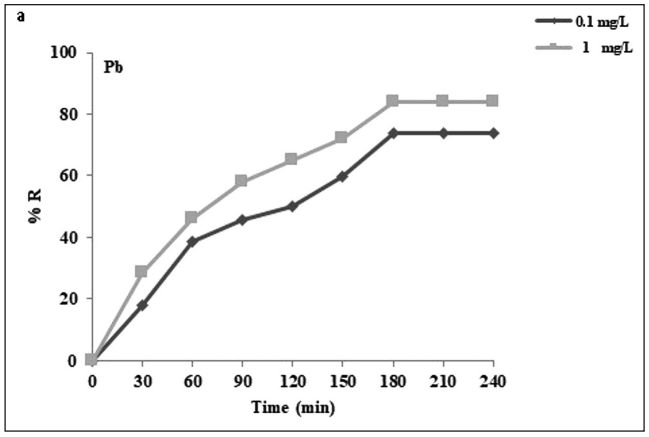

Figure 1a. Effect of time on Pb removal (%R) by dead *Lemna gibba*.

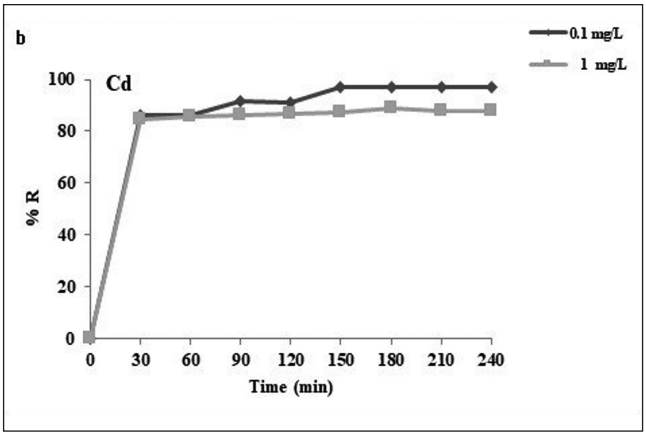

Figure 1b. Effect of time on Cd removal (%R) by dead *Lemna gibba*.

minutes and the percentage reduction remains unchanged from 180 to 240 minutes. The maximum values of 84.01% and 73.82% are obtained at 0.1 and 1 mg/L respectively. This suggests that during the second period, equilibrium is reached. Thus the equilibrium time is set at 120 minutes.

Regarding Cd (Figure 1b), the equilibrium is reached more rapidly (30 minutes) leading to a maximum removal of 93% and 88 % at 0.1 and 1 mg/L at the end of the experience.

On the other hand, the retention of Cd is reported to be larger than that of lead. Indeed, at 30 minutes, 28.66% of Pb is removed from the solution contaminated with 0.1mg/L (Figure 1a), while the removal rate of Cd reached 86.45% (Figure 1b). Similarly, in the presence of 1mg/L, Pb is removed from the solution at only 17.77%, while the percentage retention of Cd is 84.83%.

The amounts adsorbed of Pb and Cd by *Lemna gibba* powder increase with time to reach maximum values of 0.025 mg/g and 0.014 mg/g, respectively (Table 2), at 0.1 mg/L concentration. At 1 mg/L, amounts of 0.076 mg/g of Pb and 0.154 mg/g of Cd, are attached to the biosorbent.

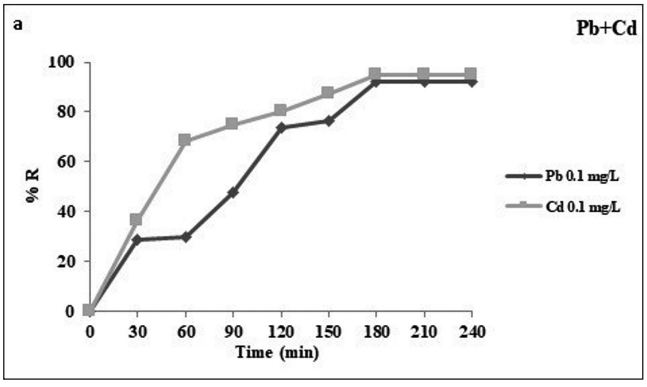

Figure 2a. Effect of time on Pb +Cd removal (%R) at 0.1 mg/L each metal by dead *Lemna gibba*.

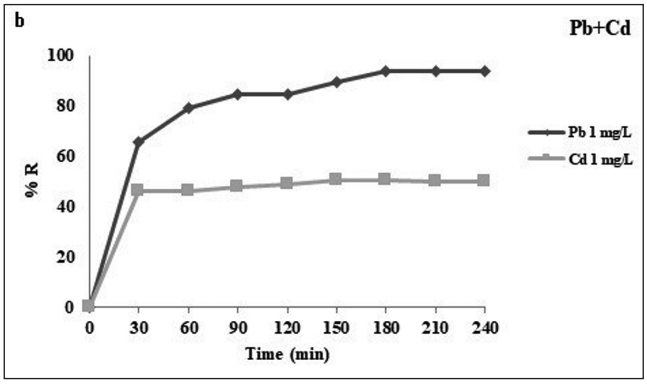

Figure 2b. Effect of time on Pb +Cd removal (%R) at 1 mg/L each metal by dead *Lemna gibba*.

Table 2. Fitting parameters of biosorption kinetic using Pseudo-first order and Pseudo-second order models**.**

		Pseudo-first order			**Pseudo-second order**		
Metal	**Concentration (mg/L)**	**R^2**	**q_e (mg/g)**	**K_1(min^{-1})**	**R^2**	**q_e (mg/g)**	**K_2($g.mg^{-1}.min^{-1}$)**
Pb_{ind}	0.1 1	0.920 0.974	0.0269 0.0772	0.015 0.011	0.982 0.982	0.035 0.095	0.357 0.132
Cd_{ind}	0.1 1	0.696 0.754	0.0085 0.041	0.020 0.026	0.999 0.999	0.019 0.154	6.333 3.926
Pb_{mix}	0.1 1	0.917 0.933	0.028 0.044	0.012 0.022	0.670 0.999	0.054 0.067	0.076 0.998
Cd_{mix}	0.1 1	0.979 0.822	0.017 0.046	0.016 0.027	0.989 0.999	0.023 0.096	0.774 1.888

In the Pb+Cd mixture, equilibrium is reached at 180 min in the presence of 0.1mg/L of each contaminant (Figure 2a). The removal of Pb and Cd is around 90%.

From 90min, the concentration of Pb and Cd in the solutions treated with 1mg Pb/L+ 1mg Cd/L changes slightly (Figure 2b). The maximum percentage of Pb removal is 86%, and that of Cd is close to 54%.

The amounts of Pb and Cd adsorbed by dried *Lemna gibba* powder from the solutions contaminated simultaneously by the two heavy metals are reported in table 2. The maximum amounts of Pb and Cd retained, are respectively 0.021mg/g and 0.017 mg/g in the mixture containing 0.1mg/L of each metal.

Lemna gibba powder retains a maximum of 0.063mg/g of Pb and 0.094 mg/g of Cd from the mixture contaminated with 1mg/L of each metal. From the results obtained, it appears that the quantities of Pb and Cd fixed on our biosorbent, from the solutions treated by the two metals individually, are very close to those retained from the mixtures.

It clearly appears that the retention of Cd and/ or Pb increases with the adsorbate-biosorbent contact time, to reach maximum values.

Table 3 – Biosorption capacities of Pb and Cd by dead *Lemna gibba*.

System	**C_0 (mg/L)**	**q_e (mg/g)**
Pb alone	0.1 1.0	0.025 0.076
Pb (Pb+Cd)	0.1 1.0	0.021 0.063
Cd alone	0.1 1.0	0.014 0.154
Cd (Pb+Cd)	0.1 1.0	0.017 0.094

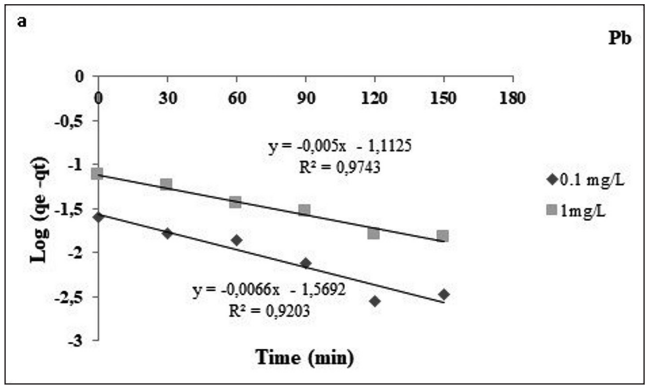

Figure 3a. Pseudo-first-order biosorption kinetics of Pbon dead *Lemna gibba*.

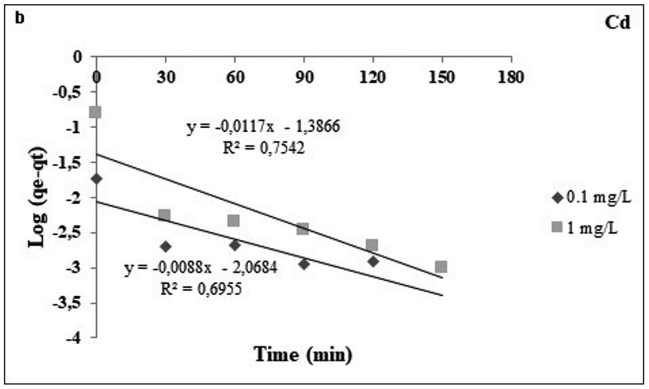

Figure 3b. Pseudo-first-order biosorption kinetics of Cd on dead *Lemna gibba*.

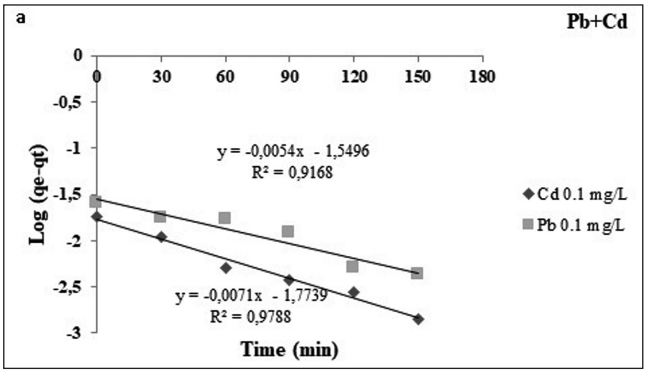

Figure 4a. Pseudo-first-order biosorption kinetics of Pb and Cd from the mixture Pb+Cd at -0.1 mg/L each metal on dead *Lemna gibba*.

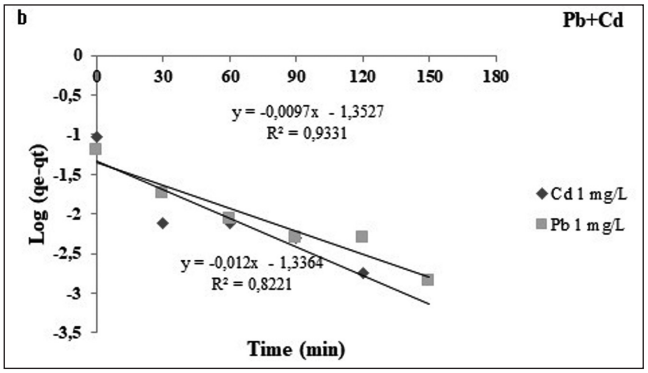

Figure 4b. Pseudo-first-order biosorption kinetics of Pb and Cd from the mixture Pb+Cd at 1 mg/L each metal, on dead *Lemna gibba*.

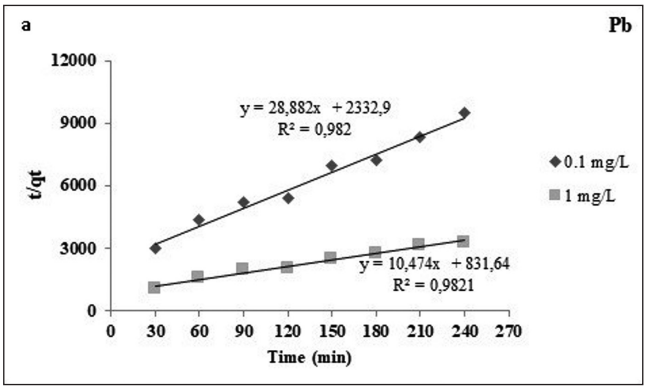

Figure 5a. Pseudo-second-order biosorption kinetics of Pb on dead *Lemna gibba*.

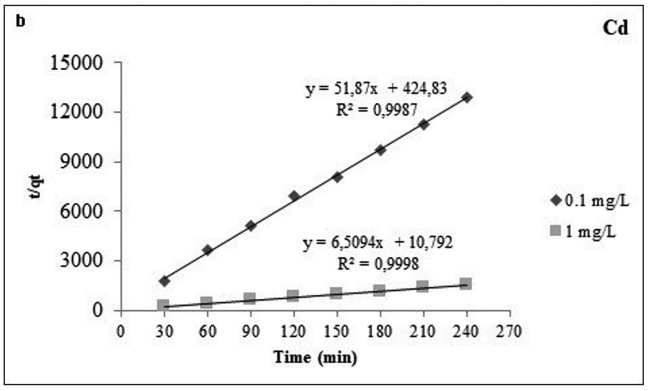

Figure 5b. Pseudo-second-order biosorption kinetics of Cd on dead *Lemna gibba*.

Modelling of biosorption kinetics

The linearized pseudo- first-order and pseudo-second order models of the sorption of cadmium and lead individually or combined onto dried *Lemna* powder at various initial concentrations are depicted in figures 3,4,5 and 6. The values obtained from the plots of the kinetic models, are shown in Table 3.

The pseudo second order model agreed better with the kinetics data of Pb sorption (Figure 5a) by dried *Lemna gibba* than the pseudo first order model (Figure 3a), with a high regression coefficient (0.982 at 0.1mg/L and at 1mg/L). Table 3 shows that the experimental q_e values (0.035 and 0.095) were quite near to the q_e values (0.027 and 0.077, respectively). In addition, the value coefficient R^2 of 0.999 suggested that Pb adsorption process follows second-order kinetics in the mixture comprising 1 mg/L of each metal (Figure 6.a). However, a straight line generated by plotting log (q_e-q_t) vs. t (Figure 4a) revealed that the pseudo-first-order equation suited the experimental findings well, yielding $R^2 = 0.917$ in the binary mixture with 0.1 mg/L each metal. The theoretical q_e values of 0.028 mg/g and the experimental data (0.021 mg/g) were almost identical (Table 3).

The pseudo-second order model for cadmium, either separately (Figure 5b) or in binary mixes (Figure 6b) showed that the correlation coefficients R^2, were found to be high (0.999). Furthermore, the experimental q_e measured are remarkably similar to those predicted by the plots (Table 3).

Effect of dry plant mass or dose

The results of the removal (R%) of lead, cadmium and their mixtures at the different amounts of the biosorbent, are shown in Figures 7a, b and Figures 8a,b.

For all the results, a considerable rise in the capacity of the biomaterial biosorption towards the contaminants is noticed with the increase of the biosorbent mass. Therefore, at the greatest mass value of the aquatic plant powder (0.5g), the retention percentages are ranging from 71% to 89%. Consequently, the optimal amount of biosorbent is 0.5 g/100mL. However, for the test performed with the mixture of 1mgCd/L+1mgPb/L, the maximum retention of 81.39% (Figure 8b) is reached for a mass of 0.25 g /100 mL.

It is well established that metal removal efficiency not only depends on the type of biosorbent but also on its quantity.

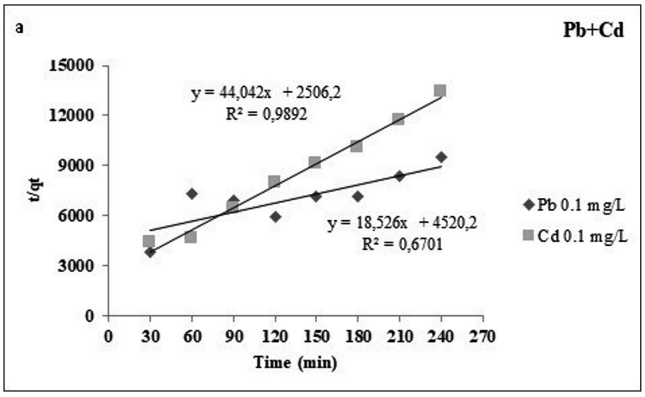

Figure 6a. Pseudo-second-order biosorption kinetics of Pb and Cd from the mixture Pb+Cd, on dead *Lemna gibba*.

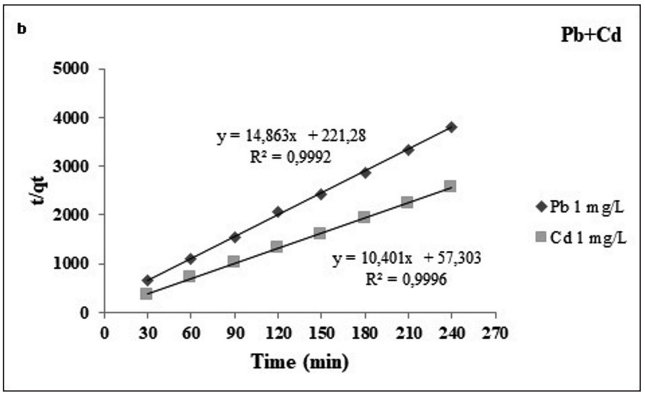

Figure 6b. Pseudo-second-order biosorption kinetics of Pb and Cd from the mixture Pb+Cd on dead *Lemna gibba*.

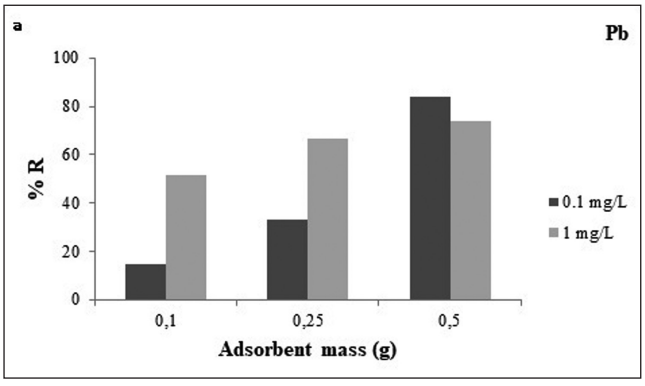

Figure 7a. Effect of the adsorbent mass on Pb removal.

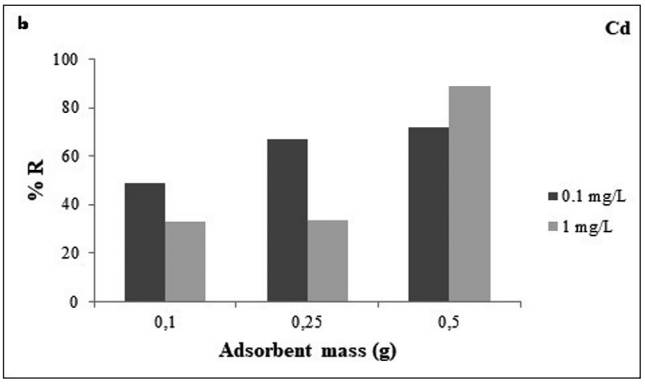

Figure 7b. Effect of the the adsorbent mass Cd removal.

Comparable findings have been recorded in the literature (Chen et al.; 2015).

Effect of contaminant concentration

Increasing the concentration of contaminants from 0.1mg/L to 1mg/L resulted in an increase in the amount of each metal adsorbed by dead *Lemna gibba* powder, either when contaminated individually or simultaneously with Pb and Cd. At equilibrium, Pb content increased from 0.025 mg/g to 0.075 mg/g and Cd content from 0.014 mg/g to 0.152 mg/g. Similarly, when the dead *Lemna gibba* is co-contaminated by Pb and Cd, the content of each metal also climbed when the concentration of each metal is raised from 0.1 to 1 mg/L.

Discussion

Biosorption process depends on the molecular structure and the functional groups of the sorbent (Gusain & Suthar, 2017). FTIR analysis of dead *Lemna gibba* powder before and after adsorption of Pb, Cd and Pb+ Cd, demonstrated the presence of numerous functional groups involved in the adsorption of the metals either individually or in binary mixtures onto *Lemna gibba*.

Various significant peaks of the dried *Lemna gibba* in the spectrum are consistent with the previous work. O-H is the peak of 3700–3200 cm^{-1} which indicates polymeric compounds. The band around 2900 cm^{-1} was usually related to the C–H stretching vibration of CH_2 (Ghasemi et al., 2014). The peak of 1600–1300 cm^{-1} described the bonding of C-H is alkyl carbonate (C-OH) (Aichour & Zaghouane-Boudiaf, 2019; Singh et al., 2018; Ibrahim & Hamed, 2018; Saleh et al., 2020).

The contact time is crucial in adsorption for the removal of metals individually or in a combination (Chen et al., 2015). At equilibrium, the curves are in the form of a plateau showing that the biosorption of the solute is maximal.

The two stages of biosorption may be explained by considering that there are a set number of active sites in a system and that each active site can adsorb a single ion. Initial metal biosorption onto the biosorbent surface will be rapid, slowing down competition to reduce the availability of active sites (Li et al., 2008).

On the dried powder of duckweed *Lemna aequinoctialis*, the equilibrium between Cd ions and the adsorbent was reached within 180 minutes (Chen et al., 2015). In a similar study, Halaimi et al. (2014) found that Cd removal efficiencies on *Lemna gibba* powder were 50% and 60% at 0.1 et 1.0 mg/L respectively and equilibrium was achieved at 240 and 120 min.

In another study (Benhima et al., 2008), the initial stage of cadmium and lead adsorption onto dry plant microparticles is completed in no more than 30 minutes, with an uptake of around 81–87% for Cd and with up to 97% Pb ion removal. When Cd (II) and Pb (II) are adsorbed onto microparticles of dried Withania frutescens plant, The equilibrium is established in 60 minutes (Chiban et al., 2012).

Cd retention is larger than lead retention, probably due to the difference in the ionic radius of the two metals. Cd radius (0.95 Å) is smaller than Pb radius (1.19 Å), thus the motion of lead by diffusion, in the liquid is slower. Therefore, the transfer of Pb ions from most of the solution to the surface of the adsorbent is less than the transfer of Cd ions in aqueous solution. (Saleh et al., 2020).

The % adsorption of metal ions from Anza wastewater followed the order of Pb (II) > Cd (II). A similar trend has been noticed in the removal of divalent metal ions (Cu (II), Cd (II), Zn (II) and Pb (II)) by other plants (Benhima et al.,2008).

The use of *Lemna gibba* in the fresh state (phytoremediation) by Aggoun et al. (2018) resulted in Pb reduction of 57% at 1mg/L. In the binary mixture Cd+Pb (Aggoun & Benmaamar, 2019), the maximum reductions are 100 % of Pb and 41% of Cd at 0.1 mg/L each metal. The removal percentages are73 % of Pb and 27% of Cd in the mixture with 1 mg/L each metal.

In several studies examining the kinetics of metal adsorption onto various adsorbents, high correlations for the pseudo-second order model have also been discovered (Karthikeyan et al., 2005; Aydin & Askoy, 2009; Hu et al., 2011; Chen et al., 2015; Halaimi et al., 2014). This revealed that cadmium adsorption was the result of a chemical interaction. It also suggested that the rate of adsorption was related to the number of vacant sites.

The quantity of each metal absorbed by dead *Lemna gibba* powder increased when the pollutants' concentration was raised from 0.1 mg/L to 1 mg/L. Thus, the increased concentration of the two ions in the aqueous medium at the beginning of the biosorption process stimulates the diffusion of the ions from the liquid to the functional group of the biosorbent (Chen et al., 2015; Deng et al., 2016).

The electrostatic attraction type interactions between the positive charges of Pb and Cd and the negative charges of the biosorption sites situated on the surface of the dead *Lemna gibba* powder might possibly explain how lead or cadmium molecules attach to one another (Halaimi et al., 2014)

The amount of Pb and Cd that dried *Lemna gibba* powder absorbed increased when pollutants were added in concentrations ranging from 0.1 mg/L to 1 mg/L, whether Pb and Cd were added separately or concurrently. Cd content, increased from 0.014 mg/g to 0.152 mg/g and Pb concentration increased from 0.025 mg/g to 0.075 mg/g at equilibrium.

It is well established that metal removal efficiency not only depends on the type of biosorbent but also on its quantity. Comparable results are reported in the literature (Chen et al., 2015)

The observed improved lead and cadmium removal efficacy could be due to the vacant sites available for uptake of Pb and Cd species upon rise in biosorbent dose.

Conclusion

A common method for eliminating metal pollution and other hazardous elements from water is biosorption by dead dried plants. The natural material employed in this

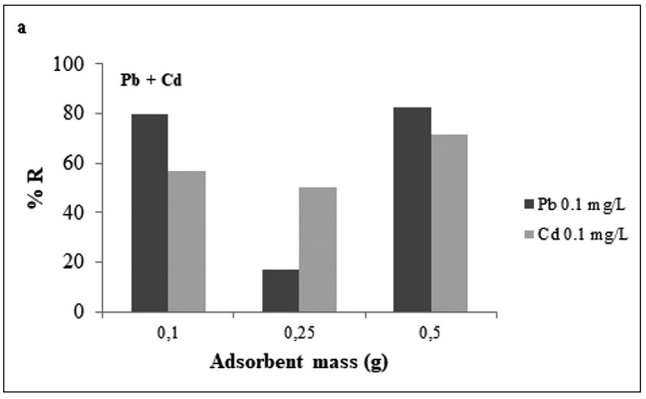

Figure 8a. Effect of the adsorbent mass on Pb +Cd removal at 0.1 mg/L each metal.

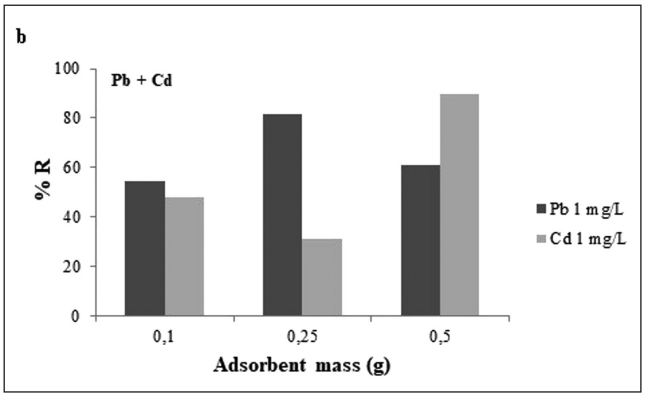

Figure 8b. Effect of the adsorbent mass on Pb +Cd removal at 1 mg/L each by dead *Lemna gibba*.

study is a good candidate as adsorbents in heavy metals removal approaches, considering the fact that this adsorbent is naturally ubiquitous and quite affordable. An effective and cheap adsorbent prepared from the dry biomass of *Lemna gibba* plant was successfully applied as biosorbent to remove highly toxic metals such as lead and cadmium either individually or in combination, from aqueous medium.

On the surface of the dead plant, FTIR analysis identified a number of relevant functional groups.

Biosorption of Pb and Cd on dead *Lemna gibba* surface increased with time and maximum adsorption achieved varied fom 73.82- 90% and 54- 93% respectively, either individually or in mixtures. The adsorption capacity of this material for cadmium and lead is of the same order of magnitude that has been found using other biosorbents or even higher than that when *Lemna gibba* was used in phytoremediation.

The pseudo second order model was found to suit Pb and Cd adsorption processes more closely than the pseudo first order model.

The use of this technology is expected to result in the efficient removal of hazardous metals, thus lowering the price of water purification with an ecological focus.

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THE PLANTS OF JERICHO. THE EARLIEST CULTIVARS BETWEEN SYMBIOSIS AND DOMESTICATION

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Abstract – Pre-Pottery Neolithic Jericho is the archaeological site in Palestine which provided the earliest archaeobotanical evidence of plant domestication. Together with an overview of finds and their historical-archaeological significance, this paper suggests considering the relationship between humans and plants at its earliest stage as a biunivocal one, as plants became an irreplaceable source of food for humans and domesticated plants could not have existed without humans.

Keywords: tell es-sultan; pre-pottery neolithic; archaeology; archaeobotany; domestication; food crops; fruit trees.

Introduction

The central core of this article is to testify through the exemplary case study, for antiquity and complexity, of Tell es-Sultan/ ancient Jericho in Palestine, how archaeology and archaeobotany can help us to understand the peculiar relationship developed between a human community and specific plants, the cultivation of which blossomed over the centuries. If we put emphasis on human initiative we talk about domestication, however if we want to have a look from the perspective of reciprocal adaptability of plants and humans we could perhaps speak of symbiosis. The excavations conducted for more than a century at Tell es-Sultan by four archaeological expeditions[1] have returned a large collection of archaeobotanical finds (Hopf, 1969; 1983; 2008; Moricca et al., 2021), that have been retrieved thanks to the abilities of the archaeologists, but above all thanks to the extraordinary climatic conditions of the site, that allowed to preserve them for millennia. This is due to the geological characteristics of the Jericho soil, to the morphology of the archaeological site, and to its paleoclimatic conditions (Mimi & Jamous, 2010; Ighbareyeh, 2019). However, this significant amount of botanical data has been only partially studied, focusing on specific periods of the ancient settlement. In the present contribution, I intend to offer a sketch summary of some salient historical-archaeological themes, with respect to which the archaeobotanical data known so far either provides curious and stimulating indications or suggests the need to further deepen the research. In this preliminary work, I focus on Pre-Pottery Neolithic, the period of the first great cultural flourish of Jericho, roughly between 11,000 and 6,000 BC, hoping that the readers of this journal will be inspired to study more in depth and extensively what the excavations of Jericho have made available, contributing to the knowledge of the site.

The first domesticated plants

That hunters and gatherers collected edible plants is quite evident although relatively difficult to prove archaeologically.

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That some of these plants are the ones that would be cultivated first is instead certain, and the excavations at Jericho, although still far from having systematically collected and simultaneously studied the paleoenvironmental and archaeobotanical data, have well demonstrated it (Hopf, 1969; 1983; 2008; Moricca et al., 2021).

At the end of the Mesolithic in the Levant, during the Natufian, when the first sedentary hunters settle on the limestone spur overlooking the spring of 'Ain es-Sultan (Kenyon, 1981, 268, 271-274, pls. 144b-145, 299a; Nigro, 2014a, 57), several plant species are brought with them to sow. Eight of these are the first domesticated founder crops (Weiss & Zohari, 2011, 237; Zohary et al., 2012, 1-2). Three cereals: barley and two types of wheat, *Triticum monococcum* L.[2] and *Triticum turgidum* subsp. *dicoccum* (Schrank ex Schübl.) Thell. (einkorn and emmer); four pulses: lentil, chickpea, pea, bitter vetch, and flax. Although they belong to different species, are annuals, and are similarly harvested, dried and processed, – cleaning, milling, possible grinding, etc.-, (Weiss & Zohari, 2011, 237). These plants were selected throughout the foothills of Western Asia in what we call the Fertile Crescent by different human communities (Zohary & Hopf, 2000; Zohary et al., 2012), among which that of Jericho appears to be one of the most precocious in the domestication of the greatest number of different species (Zohary et al., 2012, Map 1). Nevertheless, the domestication process was polyphyletic with each community or regional area specializing on specific crops.

Following the findings of the layers between Natufian/ Proto-Neolithic and Pre-Pottery Neolithic A, the first cultivated plants appear to be wild emmer (*Triticum turgidum* subsp. *dicoccoides* (Asch. & Graebn.) Thell.), wild einkorn (*T. boeoticum* Boiss.), and the small lentil (*Vicia orientalis* Beg. & Diratz.) (Hopf, 1983). Followed shortly afterwards by flax (*Linum bienne* Mill.) (Hopf, 1983 sub *L. usitatissimum* L. subsp. *angustifolium* (Huds.) Thell.) and barley (*Hordeum spontaneum* K.Koch) (Badr et al., 2000). They are crops that are practiced in relative extension, which therefore mark the definitive transition to agriculture. Within a millennium these species will be domesticated[3] and will become *T.monococcum* (Hopf, 1983, 580) and *T. turgidum* subsp. *dicoccum* (Hopf, 1983, 582; Weide, 2015, 381-424), *Hordeum vulgare* L.*, V. lens* Coss. & Germ. (Hopf, 1983, 584), and *L. usitatissimum* L. var. *usitatissimum* (Helbæk, 1959; Hopf 1983, 586) recognizable by more regular shapes and slightly larger sizes (Weiss & Zohari, 2011, 238).

Triticum monococcum and *T. turgidum* subsp. *dicoccum*, in addition to being resilient during cultivation, once harvested, and after the necessary dehusking to eliminate the glumes, could be stored for several seasons, before being consumed. These characteristics made them the main grains used to produce flour. Even though according to Maria Hopf (1983, 582), barley and wheat were brought to Jericho from Syria and Anatolia where domestication had already begun, the complete sequence of occupation in Jericho and the presence in the Natufian strata of the wild ancestor of emmer, *T. turgidum* subsp. *dicoccoides* seem to suggest that it was precisely the community settled in the Pre-Pottery Neolithic A at Tell es-Sultan that carried out this domestication. It was a centuries-long process that took place during the eleventh millennium BC and involved barley and wheats (emmer and einkorn, the latter more widespread in Jericho).

Other than cereals, an essential role in increasing the variety of the diet of Jerichiotes with proteins is played by legumes. Next to the lentil (*Vicia lens*), progressively appears the chick-pea (*Cicer arietinum* L.), the field pea (*Lathyrus oleraceus* Lam.) and the bitter vetch (*Ervilia sativa* Link.) (Hopf, 1983); the latter probably introduced in connection with the beginning of breeding of goats as fodder.

Among the cultivated plants of the first Neolithic community of Jericho, one can hardly overlook the importance of flax (*Linum usitatissimum*), whose seeds were used to obtain the precious oil used for handicrafts and constructions, for the preparation of body ointments, perfumes and foods (Pengilly, 2003) and from whose stems a resistant and malleable fiber was produced for the making of ropes, mats, bags and wicks covered in wax, useful for lighting a fire, and, only later, fabrics and textiles (Allaby et al., 2005, 63; Geyer, 2012,1). The cultivation of flax plants, that can reach the height of 1.2 m (Orendi, 2020, 63), and the processing of their products (seeds and stems) required a lot of water and a considerable workforce (not particularly specialized), from the preparation of the fields for sowing and harvesting, to the maceration, extraction and straightening of the fibers of their woody stems for textile production (Karg, 2011, 507; Shamir, 2020). Therefore, to cultivate flax, fertile arable land and water for irrigation are needed, while the subsequent processing, demands more water and well-plastered tanks for soaking the fibers, and mastabas and platforms for hammering, drying and extract them (Karg, 2011, 507). In her recent study on cultivations, by examining Egyptian papyri of the mid-sixth century of our era, Isabelle Marthot-Santaniello (2020, 113-114) points out that given these conditions, the cultivation of flax could be favorably alternated with that of wheat. In Jericho, since the Pre-Pottery Neolithic, when the waters of the spring of 'Ain es-Sultan were regularized (Nigro, 2014b, 28), the most favorable environmental conditions for the cultivation of flax arose, and at the same time the exponential growth of the population provided the necessary workforce for agriculture (Nigro, 2020, 180). The production of fibers is not yet fully demonstrated by the archaeological record until the Bronze Age. As far as archaeological finds are concerned, the carefully plastered silos brought to light in the layers of the

Pre-Pottery Neolithic were indeed used to store the precious seeds of barley, wheats and flax, the first real wealth of the Neolithic community (Nigro, 2016, 6). Some of such silos, due to the hydro-repellent quality of their plaster may have been used to soak the flax stems.
Nonetheless, in the Neolithic society of Jericho, flax has a central position, equivalent to that of barley and wheat. From the interaction of the cultivation of cereals, legumes, and flax, the first agricultural society was born. The question is: is production carried out at a family, clan, or community level?

Fruit trees

In Neolithic Jericho, fruit trees represent a very important source of sustenance since the time of hunters and gatherers, as the intake of sugars was essential for the intense physical activity of humans at the time.
Some wild fruits were fundamental for the subsistence of the first inhabitants of Tell es-Sultan. The most widespread was probably the carob tree (*Ceratonia siliqua* L.), whose bacilliform fruits, the carob pods, also called bread of St. John[4], are edible and sweet (Zohary, 2002). The dried carob seeds (*qarat* in Arabic, the 'carats'), are so light that they were later used as a unit of measurement for precious powders (spices and colors), gold and precious stones. Carob fruits are rich in sugar and give a lot of energy but are overly sweet; they are also good for animals and are easily preserved when dried.
Very similar to the carob tree is the jujube (*Ziziphus spina-christi* (L.) Willd.) (Zohary, 1973, 380-383), a shrub that to defend itself from goats has developed thorns 6-8 cm long and whose fruits, the jujube dates, were one of the favorite sweets of the first inhabitants of Jericho[5].
Other edible fruit plants available in the Jericho area are the wild pumpkin (*Citrullus colocynthis* (L.) Schrad.), the Egyptian caper (*Capparis spinosa* L. var. *aegyptia* (Lam.) Boiss.), and, while it is very common and known, the "Sodom apple" (*Calotropis procera* (Aiton.) W.T. Aiton), however, it has got lethal properties and it is not edible (but provides a fiber possibly used to make containers or fabrics) (Zohary, 1962).
Different is the case of the pistachio (*Pistacia vera* L.), a plant that was selected and grafted to eat the tasty dried fruits, and also the terebinth (*P. terebinthus* L. subsp. *palaestina* (Boiss.) Engl.) (Liphschitz & Bigger, 1990; Zohary, 1973, 135), the lentisk (*P. lentiscus* L.) and the mastic tree (*P. atlantica* Desf.) (Hopf, 1983, 588), which were exploited for the fruits, the wood and the fragrant resin (Golan-Goldhirsh, 2009, 69-70). In the steppe and around the spring, it is also widespread the *Tamarix nilotica* (Ehrenb.) Bunge., a bushy plant with disinfectant and anti-inflammatory properties (Hopf, 1983, 577).
Much more significant is the contribution provided by fruit trees that are cultivated for the first time to ensure their productivity and quality of their fruits, and where human intervention is necessary (Zohary & Spiegel-Roy, 1975; Weiss, 2015).
The first one, the fig (*Ficus carica* L.) (Goor, 1965; Hopf, 1983, 587; Lev-Yadun, 2022), is of the utmost importance, and in order to increase its productivity the inhabitants of Jericho practiced pollination and learnt the gender of individual plants. Archaeobotanical remains show that its domestication has produced local varieties since Pre-Pottery Neolithic A. The fig was a source of sugar and a reserve of yeast, essential to trigger the fermentation of fruit juices and thus enabling their preservation (Nigro & Rinaldi, 2020, 186).
The second fruit plant attested in the Pre-Pottery Neolithic A is the pomegranate (*Punica granatum* L.) (Hopf, 1983, 587; Spagnoli, 2019). The wild species, the *P. protopunica* Balf. f., characterized by marked vertical ridges and overall small dimensions, in Jericho was transformed into the 'apple of paradise'[6]. A golden red apple, with 613 seeds, a symbolic prime number for the Bible; a fruit with healing, antiseptic, anti-inflammatory and aphrodisiac properties, whose astringent juice could be used in the precipitation of milk rennet and in the fermentation of fruit as well as to produce wine, symbolic of life and fertility[7]. The pomegranate (*P. granatum*) – perhaps as early as the Pre-Pottery Neolithic – is in fact a symbol of fertility and beauty (Abram, 2009).
The third fruit tree cultivated in Jericho since the Neolithic is the date palm (*Phoenix dactylifera* L.) (Goor, 1967; Hopf, 1983, 589), which represented another significant source of extremely precious energy for all those who ventured in the desert, where dried dates, that could be kept for a long time (Chao & Krueger, 2007, 1080), could support travelers and be planted in oases, – the actual palm needs water (Longo, 2001, 617). But the secret was to have understood the mechanism of pollination and to have the flowers available, which in the climate of Jericho was more than easy. The date palm with its very long leaves (up to 5 m) with their long stems with sharp, hard and pointed ends and its fibrous but resistant wood also offered a useful building material, suitable to cover the first huts of the Neolithic village and to innervate the adobe walls. It is curious that this palm, *P. dactylifera,* was given the name of those who marketed it in the first millennium BC, the Phoenicians, just like the other great tree-symbol domesticated in Jericho in the Neolithic, the pomegranate (*Punica granatum*) (Nigro & Spagnoli, 2018, 59).
Last of the series of fruit trees is the almond tree (*Prunus dulcis* (Mill.) D.A. Webb) (Zohary & Hopf, 1993) which also appears among the most common and essential plants for the diet of the Pre-Pottery Neolithic. Domestication, in this case, counteracted the presence of hydrogen cyanide in

the seed. The drupes are not edible, but the seed is, although in some cases it can develop amygdalin, which is toxic. Almonds could provide an important protein intake, but to consume them without harm, they needed to be roasted first. Since the plant is not self-fertilizing, domestication indicates the acquisition of practical knowledge that was certainly sophisticated for the Neolithic. The retrieval of 1 mm thick shells may not be sufficient to tell whether these were already domesticated specimens, but the presence of almonds in the archaeological record of Neolithic Jericho is nonetheless significant.

Finally, the vine (Goor, 1966a; Zohary, 1995) and the olive tree (Goor, 1966b; Eitam & Heltzer, 1996; Barazani et al., 2023), whose cultivation must take place on a large scale to produce a significant economic effect, were instead the result of the first urban society of the Bronze Age and, therefore go beyond the scope of the present study, and require a dedicated one. Nevertheless, grapes and olives have been found in the archaeological contexts of Neolithic Jericho.

Aromatic and medicinal plants

A separate chapter is that of aromatic and medicinal plants very common and already known from the Paleolithic and that grew abundantly in the region of Jericho: mallow (*Malva sylvestris* L.), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.), sage (*Salvia* L.), rosemary (*S. rosmarinus* Spenn.) and finally the so-called "rose of Jericho" (*Anastatica hierochuntica* L.). Other useful herbs, such as calendula (*Calendula officinalis* L.), oat (*Avena sativa* L.), borage (*Borago officinalis* L.), poppy (*Papaver somniferum* L.) and rose, special for pollinating insects, are witnessed in pollen residues (Hopf, 1983, 591).

Another plant certainly exploited at least since the Neolithic that grows abundantly in the Jericho area is henna (*Lawsonia inermis* L.) and a purple flower (*Crocus sativus* L.) with whose dried stigmas a spice is produced, the first that men have cultivated, as well as harvested and imported: saffron. The coloring property of this plant added a symbolic component (Martinez, 2022, 19).

Tubers

Tubers and roots, which were the result of the experience of the Natufian gatherers, are equally present as the carrot (*Daucus carota* L.) and the beetroot (*Beta vulgaris* L.) (Zohary, 1962), while the onion will be selected in Egypt later. This kind of plant remains are very rare to be found and the possibility of properly defining their contribution to the Neolithic diet of the Jericho inhabitants is scarce.

Timber trees

Firewood or construction timber is a very rare material in the Jericho region. Several shrubs that grow in the steppe surrounding the oasis (that during the Neolithic it's irrigated only minimally), can take on an arboreal appearance (Fahn et al., 1986). In addition to the different species of *Pistacia* that are endemic and the other fruit trees already mentioned, the wood available to the inhabitants of Jericho came exclusively from the poplars (*Populus* L.) that grew along the Jordan River (Zohary, 1962, 165), from tamarisks (*Tamarix tetragyna* Ehrenb.) (Hopf, 1983, 577; Western, 1971), and from acacias (Red Acacia*, Vachellia seyal* (Delile) P.J.H.Hurter)[8] that could reach a maximum height of 10 m, with beams no longer than 4-5 m.

It is evident that these plants were exploited, but they were also fully integrated into the daily life of the inhabitants of Jericho.

Conclusions

The results of the excavations at Tell es-Sultan/ancient Jericho show how the first definitively settled community in Pre-Pottery Neolithic A found numerous sources of subsistence in plants and began to cultivate them intensively. The archaeobotanical data and the specificity of domesticated plants and their cultivation lead one to ask some basic questions: were there farmers who specialized in the primary production of grains and legumes and others who devoted themselves to fruits and aromatic and medicinal plants? Are there plants (particularly tubers and vegetables) that escape the archaeological record – such as cauliflowers, which sources would like to have originated in Cyprus (Maggioni, 2015, 51), but which may also have originated in the alluvial valleys of the great rivers of the Near East -? What may be the indicators of domestication of fruit trees?

Other plants, small in size, are not attested: but is this sufficient to say that they were not used by the inhabitants of Jericho? Sesame (*Sesamum indicum* L.), for example, native to Africa (Mehra, 2000), may have reached the Jordan Valley as early as the Neolithic period.

The process of domestication represents an extraordinary phenomenon particularly for those species whose modifications we are able to describe: the eight Neolithic founder crops represent the abilities of the human community of Jericho to select seeds, store them and pass them on through generations

(at least 40 for 1,000 years). Territory, human community, and cultivar became increasingly integrated, not only as an anthropological and botanical process, but a cultural phenomenon. The culture of the seed that needs to be harvested, preserved, sown and allowed to blossom and grow becomes – for the first time in history – a conceptual and cultural palimpsest on which to build the development of the human community. Thus, humans can be said to be "botanizing," desiring to resemble plants and nature in order to seize and develop their fruitfulness, resilience and generosity. That is why I believe we can speak of symbiosis, of living together.

Even if many points remain obscure, and I do not think that this brief note has helped to clarify them all, I hope that readers more experienced than I will be interested in the issues discussed above and will further develop the research.

Aknowledgements

I am not sure that my contribution can find a worthy place in a scientific venue as high as the one that hosts it and I am grateful to the Editor for accepting it, even if the setting of the text and the study that generated it are perhaps not exactly orthodox from the point of view of an archaeobotanist. I want to thank Claudia Moricca for having re-read the text (the responsibility for what has been written is totally mine).

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Sitography

https://sites.google.com/uniroma1.it/sapienzatojericho.

Notes

1 The Austro-German mission (1907-1909) directed by Ernst Sellin and Carl Watzinger (Sellin & Watzinger, 1913); the first British mission (1930-1936) directed by John Garstang (Garstang, 1927; 1930; 1931;1932a-b; 1933; 1934; Garstang et al., 1935; 1936; Garstang & Garstang, 1948); the second British mission (1952-1958) directed by Kathleen M. Kenyon (Kenyon, 1951; 1957; 1960; 1965; 1981; Kenyon & Holland, 1982; 1983); and the Italian-Palestinian mission (1997-2022) (Nigro, in press); [major excavation reports have been published: ROSAPAT 1,2,4,5,7,13 and more than 100 articles on refereed journals (for the latest see Nigro, 2023); for the complete and updated bibliography of «La Sapienza» Expeditions to Palestine & Jordan see https://sites.google.com/uniroma1.it/sapienzatojericho].

2 The nomenclature of the species follows the *International Plant Names Index* (IPNI; https://www.ipni.org/) and the accepted taxa agree with *Plants of the World Online* (POWO; https://powo.science.kew.org/)

3 Features of domestication can be summarized as following: ear shattering in cereals, pod's indehiscence in legumes and indehiscence of capsules in flax. In all cases, the seeds are retained by their containers in the cultivated plants. Another distinctive element of cultivated plants compared to wild ones are the size of the seeds (Weiss & Zohari, 2011, 238).

4 St. John the Baptist, a hermit in the wilderness of Judah (near Jericho), evidently ate it (Matt. 3:11, 11:1-11; 14:1-12 and synoptics).

5 Some authors suggest that the fermented jujube juice was the drink usually consumed by the Lotophagi, the inhabitants, according to some, of the island of Djerba, narrated in Book IX of the Odyssey. The jujube broth, or jujube, is sweet and cloying: "andare in brodo di giuggiole", in Italian, lit. "getting into jujube broth", means to gloat out of vanity.

6 One of the hypotheses is in fact that the "apple" of Genesis 1 that Eve picks from the tree of the knowledge and offers to Adam, is not really an apple, but a *malon* known from the Greek version of the Septuagint, that means simply "the fruit". It could therefore also be a pomegranate, among the first domesticated apple-shaped fruits in the Near East.

7 (Nigro & Spagnoli, 2018, 49) This same apple embellished with a seven- or nine-pointed crown was chosen by the ancient kings of the Levant as the finial of their ivory and gold sceptres. A plant that for Pharaoh Tuthmosis III, who conquered Palestine and Syria on behalf of his mother, Queen Hatshepsut, was the most beautiful of his "Syrian garden" that he had represented carved in Karnak, in front of Thebes, Egypt.

8 Otherwise known as *Faidherbia albida* (Delile) A.Chev., in Hebrew *shittah* or plural *shitthim,* with whose wood the Ark of the Covenant it is said to be made of in the Exodus (Ex. 37:1: "Bezalel made the ark of acacia wood—two and a half cubits long, a cubit and a half wide, and a cubit and a half high").

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Research articles: substantial, original research contributions on any aspect of coenology and plant ecology. The main body of the text (excluding tables and references) should not exceed 6000 words, and eight figures and tables.

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Manuscripts must be written in English, and should conform to standard rules of English grammar and style. Text should be written in MS Word, double-spaced with settings for A4 (210 x 297 mm) paper with wide margins. Use Times New Roman font, pt-size 12 (symbol palette for additional characters). Lines and pages should be consecutively numbered.

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Acknowledgements: Brief list of individuals who provided help during the research. References to research projects/funds can be quoted here.

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Pignatti S., 1982. Flora d' Italia, 3 Vol., Edagricole, Bologna.

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ANNOTATIONS

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