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EVALUATION OF WHEAT GERMPLASM FOR RESISTANCE TO RUSTS

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ABSTRACT

Wheat is the most important food grain in Georgia. Rusts are major threat to wheat production all over the world including Georgia. Wheat sown area (46.5kg/ha) and yield (2.1t/ha) are much lower than in other countries. Unfortunately, wheat produced in Georgia meets only 10-15% of local demand. To improve the wheat productivity, CIMMYT in the frame of the International Winter Wheat Improvement Program distributes different international nurseries globally. Since 2000, this network has been providing the Georgian national breeding program with numerous nurseries comprising high yielding advanced breeding lines. As a result of regional testing of these nurseries, seven varieties with broad adaptation to range location have been released in Georgia. The objective of this study was to evaluate the sources of resistance to all three rusts in 85 wheat entries of IN-20RUST-SET under natural and artificial infections. Testing of IN-20RUST-SET germplasm obtained from CIMMYT was conducted at the research field of Institute of P&B during 2020-2021. The results of greenhouse assessment of wheat entries at the seedling stage under artificial infection revealed that 21entries were resistant, 30 and 27 entries were moderately resistant, 26 and 20 - moderately susceptible and five entries- susceptible to leaf rust and stem rust, respectively. The results of field assessment under natural infection of rusts showed that the majority of the genotypes were moderately resistant to leaf rust and stem rust. Natural infection of stripe rust was very low in the research years. Assessment of genotypes with resistant reaction will be continued under heavy infection of rusts.

Keywords: *Wheat, genotypes, leaf rust, stem rust, resistance.*

INTRODUCTION

Wheat plays very a important role in global food security. Wheat has historically been the major food crop in Georgia, and it is a major cash crop and has no practicable alternative in crop rotations, especially in the dryland areas in East Georgia (Lashkhi et al., 2014). Unfortunately, wheat sown area and yield over the past 5 years averages 46.5 thousand hectares and 2.1 t/ha, respectively, and wheat

produced in Georgia meets only 10-15% of local demand that threats food security of the nation. Wheat diseases are the most important constraints to wheat production (GeoStat, 2021). Therefore, identification and promotion of improved resistant varieties is one of the most efficient means to strengthen grain production in the country, which is the main internal priority of the Georgian agricultural sector. To improve the productivity of winter wheat, the International Maize and Wheat Improvement Center (CIMMYT) in the frame of the International Winter Wheat Improvement Program (IWWIP) develop and distribute the different International Nurseries globally to over collaborators in different countries (including Georgia). Since 2000, this network has provided the national breeding program with numerous nurseries comprising high yielding advanced breeding lines. As a result of the regional testing of these nurseries seven genotypes as varieties with broad adaptation to a range location have been released in Georgia (Morgounov et al., 2019]. The most distributed and harmful among wheat diseases are wheat rusts in Georgia. Protection of wheat from rust diseases has very special significance for the Caucasus, which is one of the origins of wheat and its pathogens having evolved together (Zhukovsky, 1973). Presence of alternate host-plants, wild cereals – infection reserves, and optimal climatic conditions provide for stable development of rusts and consequently, severe crop losses.
The objective of this study was to evaluate and identify sources of resistance to all three rusts in 85 winter wheat genotypes of IN-20RUST-SET.

MATERIALS AND METHODS

Field trials. The field trials were carried out at the experimental plot of Institute of Phytopathology and Biodiversity (5 m above sea level) during two (2019-2020 and 2020-2021) growing seasons. The tested International Wheat Rust Nursery (IN-20RUST-SET) consisted of 85 wheat genotypes originating from different country's breeding programs was obtained from the IWWIP, CIMMYT. The tested genotypes were hand-planted in 3 rows with one-meter length spaced 20 cm apart at a rate 120 seeds per meter, (Singh, 2006). Two rows of standard variety - Bezostaya 1 and universal susceptible variety Morocco were planted within the screening material after every 20th entry to enhance inoculum pressure. Measurement of rusts incidence and severity in wheat genotypes under natural conditions during each of the two growing seasons was conducted according to international methodology. Observation on host response was recorded according to Roelfs et al. (1992) and the severity of disease was recorded using the international scales specified for rusts as % of rust infection on the plants according to the modified Cobb's Scale (Peterson et al., 1948). The host plant response(TR) to the rusts was assessed using the following grades: 'R' to indicate resistance or miniature uredinia;'MR' to indicate moderate resistance, expressed as small uredinia; "MS' to indicate moderate susceptible, expressed as moderate size uredinia somewhat smaller than the fully compatible type, and "S' to indicate full susceptibility. Severity (%) was estimated for whole plants, based on the proportion of the flag leaf surface area infected by rust. The incidence of the rusts was assessed as the proportion of infected plants versus total plants assessed. Incidence and severity of rusts were recorded three times 7-10-day intervals after the appearance of the first disease symptoms. Disease severity and host response data were combined in a single value called the coefficient of infection (C.I.) what was calculated by multiplying the disease severity and a constant value for host response. These values of host response were: for immune = 0.0, R = 0.2, MR = 0.4, MS = 0.8, MR- MS = 0.6 and S = 1.0 (Stubbs et al., 1986).

Greenhouse seedling tests. Assessment of genotypes at the seedling stage was conducted in 2020-2021under artificial inoculation by using prevailed races of leaf rust (LR) and stem rust (SR). Wheat genotypes were sown into 9 cm diameter plastic pots in three replications and grown in the greenhouse conditions at 20-22 ^{0}C. Urediniospores of LR and SR were multiplied by using the susceptible cultivars Morocco and Thatcher. 3-8 days seedlings (at 1-2 leaf stage) of the tested genotypes were inoculated with water-spore suspensions by spraying of each single pustule isolate and placed in a dew chamber overnight. After 24hour infected seedlings were transferred to the greenhouse under temperature within the range of 20^{0}C - 28^{0}C. Twelve to fourteen days after inoculation, plant reactions (TR) were scored using the (0-4) Mains scale (Long, Kolmer, 1989; Jin et al., 2008).

RESULTS AND DISCUSSION

Observation of the experimental field showed that the first symptoms of stripe rust appeared on several genotypes (Morocco, Bezostaya and Seri) during the last week of May, the rest of the entries were free from stripe rust. In mid-June leaf rust and stem rust pustules with infection types "3" were episodically found on the forty and fourteen genotypes, respectively. The next records were done in the end of June and in the mid of July. Rust incidence and severity in both years were low: the severity of leaf rust and stem rust were between 1MS-30MS excluding the genotype N47 which showed susceptible reactions with high severity (60S). Also, relatively high severity (40S-60MS) of leaf rust and stem rust was indicated on varieties: Morocco (susceptible check), Bezostaya and Seri in both years.

The results of field assessment revealed that the main parts of tested genotypes had resistant and moderately resistant reaction to all three rusts. Particularly, fifteen and fourteen entries showed resistance, thirty-two and forty-three entries showed moderate resistance to leaf rust and stem rust, respectively. The moderately susceptible reaction to leaf rust and stem rust was scored on thirty-nine and thirteen genotypes, respectively. Only one genotype VEE#8//JUP/BJY/3/ F3.71/TRM/4/2* (N47) showed susceptibility to leaf rust in the field and one entry had combined MR-MS reaction to leaf rust. Nearly all of resistant entries had very low values of CI (0.2-0.5) and AUPDC (less than 10.0) are the best genotypes with very high levels of resistance, CI of susceptible genotypes varied between 0.8- 48, only one genotype had high CI - 48 (Table 1).

In accordance with the results of the seedling tests, eighteen and eleven genotypes were resistant (R), twenty-three and twenty-one genotypes were moderately resistant and twenty-seven and thirty-three genotypes were moderately susceptible to leaf rust and stem rust, respectively. Only three entries (N17, N66, N76) had susceptible reaction to leaf rust at the seedling stage (Table 1).

Table 1. The host plant reaction of wheat genotypes to leaf rust and stem rust at the seedling and adult plant stages

N	Name of genotypes	Origin	Leaf rust		Stem rust	
			TR in seedlings	TR and CI in adult plants	TR in Seedlings	TR and CI in adult plants
1	BEZOSTAYA	RUS	MS	30 MS/24	MS	10 MS/8
2	SERI	MEX	MS	5MS/4	MS	5MS/4
3	**MOROCCO**		S	20MS/16	MS	20MS/16
4	NACIBEY	TCI-ESK	MS	5MS/4	MRMS	1MS/0.8
5	DI09016	FR	MS	1MS/0.8	MSS	1MS/0.8
6	RE08030	FR	MS	5MR/2	MS	5MR/2
7	MV NEMERE	HUN	R	R	R	R
8	KRAJCAR	HUN	R	R	MR	R
9	MV-PANTALIKA	HUN	R	R	MS	R
10	GRK79/KKTS	MEX	MR	5MR/2	MR	5MR/2
11	KUPAVA/CHAPIO	MEX	MS	1MS/0.8	MS	5MR/2
12	INTENSIVNAYA/KUKUNA	MEX	MR	5MR/2	MRMS	5MR/2
13	DORADE-5/3/SUNCO.6/FRAME//PASTOR/4/	MX-TCI	MR	5MR/2	MR	5MR/2
14	MERC/4/BJY/COC//PRL/BOW/3/FRTL/	MX-TCI	MS	5MR/2	MR	5MR/2
15	MT.DESC.1E-308WM97-98/TUKURU	MX-TCI	MR	5MR/2	R	5MR/2
16	DORADE-5/11/CROC_1/AE.SQUARROSA	MX-TCI	MR	10MS/8	R	10MS/8
17	ALPU01/4/338-K1-1//ANB/BUC/3/KIRGIZ	MX-TCI	S	5MR/2	MS	5MR/2
18	FGMUT213	ROM	MR	1MR/0.4	MS	1MR/0.4
19	AJVINA	RUS	MR	1MR/0.4	MR	1MR/0.4
21	SARVAR	TAJ	R	R	MR	5MR/2
22	BLUEGIL-2/BUCUR//SIRENA	TCI	MS	1MS/0.8	MR	1MS/0.8
23	AU/3MINN//HK/38MA..9-18-3/HBF0435//2180	TCI	R	R	MR	R
24	STAR/BWD//ATAY/GALVEZ87	TCI	MR	5MR/2	MR	5MR/2
25	8229/OK81306/8/AGRI/BJY//VEE/6....	TCI	R	5MR/2	MR	5MR/2
26	TREGO/JGR 8W//DORADE-6	TCI	MS	10MS/8	MS	1MS/0.8
27	ES14/SITTA//AGRI/NAC/5/TRAP#1	TCI	MR	5MR/2	MR	5MR/2
28	ND643/2*WAXWING/4/TAM200/	TCI	MR	5MR/2	MR	5MR/2
29	AGRI/NAC//KAUZ/3/CH75479/SAR	TCI	MR	5MS/4	MR	1MR/0.4
30	ST.ERYHTR894-07/3/KIRITATI//	TCI	MR	5MR/2	MR	1MR/0.4
31	PYN*2/CO725052/3/KAUZ*2/YACO	TCI	MS	5MS/4	MS	1MR/0.4
32	PYN/BAU//ATTILA/4/ID800994.W/VEE//	TCI	MS	5MS/4	MS	1MR/0.4
33	PANTHEON/BLUEGIL-2/5/AGRI/BJY//	TCI	MS	1MS/0.8	MS	1MR/0.4
34	HEILO/4/CROC_1/AE.SQUARROSA....	TCI	MR	5MR/2	MR	1MR/0.4
35	ZNAKHIDKA/EKIZ	TCI	MS	1MS/0.8	MS	1MS/0.8
36	MV-BERES/EKIZ	TCI	MR	5MR/2	MR	5MR/2
37	F498U1-1021 / BOEMA/3/KS96HW94//	TCI	MR	5MR/2	MR	1MR/0.4
38	ZIYABEY 98/4/KS90175-1-2/CM112793//..	TCI	MR	5MR/2	MR	5MR/2
39	CRINA/BONITO-37	TCI	MR	10MS-MR	MR	10MR/4
41	T98-9//VORONA/HD2402/5/AGRI/BJY//	TCI	MR	1MS/0.8	MR	1MR/0.4
42	BLOYKA/3/AGRI/NAC//KAUZ	TCI	MR	5MR/2	MR	5MR/2
43	CHEN/AE.SQUARROSA(TAUS)//	TCI	MR	5MR/2	MR	1MR/0.4
44	TJB368.251/BUC//WEAVER/3/	TCI	MS	1MS/0.8	MS	1MS/0.8
45	ESPADA/KARAHAN	TCI	MR	5MR/2	MR	5MR/2
46	KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/	TCI	MR	5MR/2	MR	1MR/0.4
47	VEE#8//JUP/BJY/3/F3.71/TRM/4/2*	TCI	MS	60MS/48	MR	1MR/0.4

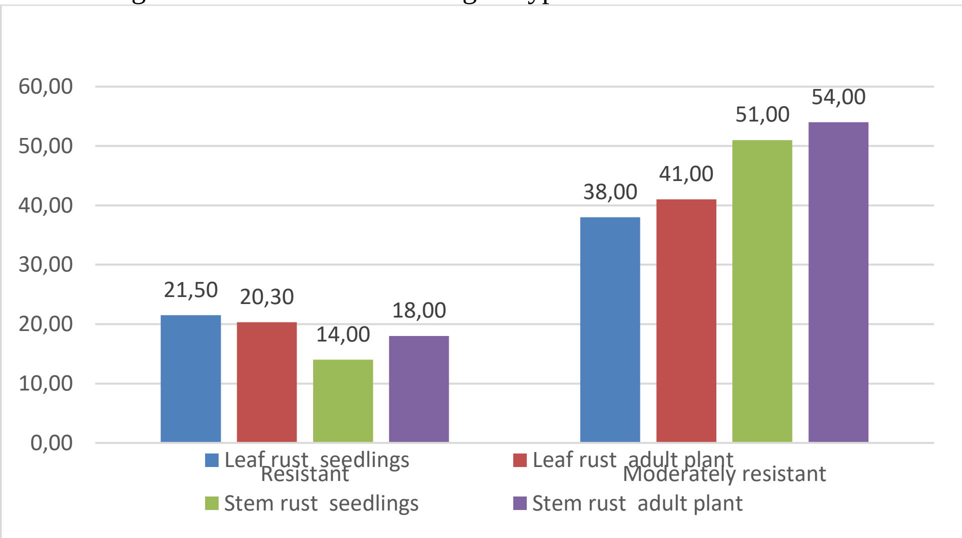
48	WBLL1*2/KIRITATI//BILLING(N566	TCI	MR	1MS/0.8	MR	1MR/0.4
49	DRAGANA/KINACI97	TCI	MS	1MS/0.8	MS	1MS/0.8
50	GONDVANA/6/53/3/ABL/1113//K92/	TCI	MR	10MR/4	MR	1MR/0.4
51	FRTL//AGRI/NAC/3/BONITO-36/4/	TCI	MS	1MS/0.8	MR	1MR/0.4
52	105/3/NE70654/BBY//BOW"S"/4/	TCI	MR	5MR/2	MR	1MR/0.4
53	338-K1-1//ANB/BUC/3/GS50A/4/059E//	TCI	MS	5MR/2	MS	5MS/4
54	ZNAKHIDKA/EKIZ	TCI	MR	5MR/2	MR	5MR/2
55	VORONA/HD2402/3/RSK/CA8055//	TCI	MR	10MR/4	MR	1MR/0.4
56	VORONA/OPATA//PYN/BAU/5/AGRI	TCI	R	R	R	R
57	87-461 A 63-555//SAULESKU #26/	TCI	MS	10MS/8	MS	1MS/0.8
58	OBRII/DNESTREANCA25//ILICIOVCA	TCI	MS	1MS/0.8	MS	1MS/0.8
59	ERITR 9945/DORADE-6/3/NEMURA/	TCI	MS	1MS/0.8	MS	1MS/0.8
61	PICAFLOR/3/KS82W409/SPN//TAM	TCI	MS	1MS/0.8	MS	1MS/0.8
62	MINO/5/REH/HARE//2*BCN/3/CROC	TCI	MS	5MR/2	MS	5MR/2
63	DANPHE #1/6/CA8055/4/ROMTAST	TCI	1MSMR	5MR/2	MR	5MR/2
64	TREGO/BTY SIB/4/338-K1-1//ANB	TCI	R	R	MR	R
65	KROSHKA/GONDVANA	TCI	MS	20MS/16	MS	5MS/4
66	AGRI/NAC//ATTILA/3/DORADE-6	TCI	S	20MS/16	MR	5MR/2
67	KUV/LJILN//ORACLE/PEHLIVAN	TCI	MS	1MS/0.8	MR	1MS/0.8
68	9852.1//ERYT1554.90/PEHLIVAN	TCI	MR	5MR/2	MR	5MR/2
69	**9852.1//ERYT1554.90/PEHLIVAN**	TCI	MR	5MR/2	MR	5MR/2
70	BILLING(N566/OK94P597)	USA	R	R	R	R
71	CO07 W245	USA CO	MS	1MS/0.8	MS	1MS/0.8
72	KS970187-1-10/KS031027-FHB~	USA-KS	R	R	R	R
73	FARMEC/KS990160-4-~5	USA-KS	MS	1MS/0.8	MS	1MS/0.8
74	KS980191-1-7/W04-417//ARMOUR	USA-KS	R	R	R	R
75	KS980554-12-~9/KS020363WM~	USA-KS	R	R	R	R
76	MNCH/ATTILA//TAM 400/3/N87V106/	USA-OK-TCI	S	5MS/4	MS	5MS/4
77	ATTILA*2/PASTOR//OK95553/OK92403..	USA-OK-TCI	R	R	R	R
78	KAMB1*2/KIRITATI//BIG DAWG/	USA-OK-TCI	R	R	MR	R
79	WBLL1*2/KIRITATI/5/T67/JGR 'S'//	USA-OK-TCI	R	R	MR	R
81	PFAU/MILAN/3/SKAUZ/KS94U215//	USA-OK-TCI	R	R	R	R
82	WBLL1*2/TUKURU//BILLINGS	USA-OK-TCI	R	R	R	R
83	PVN//CAR422/ANA/5/BOW/CROW//...	USA-OK-TCI	MR	5MR/2	MR	1MR/0.4
84	**NI12702W**	USA-UNL	-	-	-	-
85	SERI	MX	MS	10MS/8	MS	5MS/4

Thus, assessments of introduced wheat germplasm to rusts under natural and artificial infection showed that 21.5% and 14% of the tested entries were resistant, 38% and 51% - moderately resistant to leaf rust and stem rust, respectively, at the seedling stage, 20.3% and 18% of the entries were resistant and 41% and 54% -

moderately resistant to leaf rust and stem rust, respectively, at the adult plant stage (Figure 1).

All IWWIP wheat germplasm are evaluated under leaf rust, stem rust and stripe rust natural epidemic conditions and artificial rusts infection before being distributed to wheat breeding communities. According to results obtained in Izmir research station, 50% of tested genotypes of this nursery were resistant and moderately resistant to leaf rust, stripe rust and stem rust, 35% of genotypes showed MS and S reaction and 10-15% - intermediate reaction in 2017-2019 field tests (unpublished data). These data are in agreement with our research results.

Figure 1. Resistance of wheat genotypes to leaf rust and stem rust



Forty genotypes from this nursery were evaluated to stripe rust under artificial infection at the seedling and adult plant stages in Iran (Koc et al. 2023) and like our results, the majority of entries were also resistant and moderately resistant to pathogen in both stages. Over the last 20 years, numerous breeding nurseries comprising high-yielding advanced breeding lines introduced from CIMMYT and ICARDA were evaluated under diverse environments of Georgia and several varieties were selected. (Natsarishvili et. al., 2016; Sikharulidze et. al., 2015; 2013).

CONCLUSION

Thus, 59.5% and 65% of the tested entries of introduced wheat germplasm were resistant to leaf rust and stem rust, respectively, at the seedling stage. 61.3%, 72.1% and 94% of the entries were resistant to leaf rust, stem rust and stripe rust, respectively, at the adult plant stage. These lines and cultivars can be included in national breeding programs for further ecological and agronomic assessment.

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ASSESSMENT OF THE SUSTAINABILITY OF NEGLECTED AND UNDERUTILISED CROP SPECIES: SUSTLIVES APPROACH

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ABSTRACT

There are different approaches and frameworks for the assessment of sustainability in agriculture and food systems, but only a few of them focus on crops. This gap is even more evident when it comes to the so-called neglected and underutilised species (NUS). To bridge this gap, the present paper describes an approach for the assessment of the environmental, social and economic sustainability of NUS developed within the project SUSTLIVES (Sustaining and improving local crop patrimony in Burkina Faso and Niger for better lives and ecosystems). The indicators identified through a literature review, based on the Web of Science, have been integrated with other indicators used for sustainability evaluation in the agri-food sector. Based on that, a sustainability assessment approach (list of indicators, evaluation methods and units; scoring system and mode of aggregation of scores; reference crops for the selected NUS) was developed. Then, two workshops were organized in Niamey (January 2023) and Ouagadougou (February 2023) to validate the sustainability assessment approach. The validated sustainability assessment matrix contains 27 indicators divided into different themes covering the three dimensions of sustainability: environmental (environmental integrity, agronomic performance and productivity), social (cultural importance and relevance, nutritional quality and diversity, employment, equity and accessibility) and economic (competitiveness, profitability). A scoring system was proposed for each indicator; from 0 (unsustainable) to 10 (very sustainable) with 5 corresponding to the sustainability benchmark value. Besides sustainability assessment, the developed approach allows selecting the NUS with the highest potential in view of their promotion and the development of their value chains.

Keywords: *orphan crop, NUS, indicator, scoring system, sustainability benchmark.*

INTRODUCTION

Sustainability in agriculture and food systems can be assessed using different approaches and frameworks (Alaoui et al., 2022). These include Value Chain Analysis for Development (Fabre et al., 2021), Common agricultural policy performance indicators (European Commission, 2022), indicators described in SUSAGRI (Sustainable development in agriculture) project (Yli-Viikari, 1999), OECD Compendium of Agri-environmental Indicators (OECD, 2013), Response-Inducing Sustainability Evaluation (RISE) framework (Häni et al., 2003), Multi-attribute Assessment of Sustainability of Cropping Systems (MASC) (Sadok et al., 2009), Land Degradation Assessment in Drylands (LADA) framework (Nachtergaele et al., 2010), Sustainability Monitoring and Assessment RouTine (SMART) framework (Landert et al., 2020), Public goods (PG) framework (Gerrard et al., 2012), Farmer Sustainability Index (FSI) (Gayatri et al., 2016), Sustainability Assessment of Farming and the Environment (SAFE) framework (van Cauwenbergh et al., 2007), SALCAsustain method (Roesch et al., 2021), and *Indicateurs de Durabilité des Exploitations Agricoles* (IDEA)/Farm Sustainability Indicators method (Zahm et al., 2006). However, the assessment is usually not on the crop level and frameworks relate to the agricultural sector, countries, value chains, farms or others. This gap is even more evident when it comes to the so-called neglected and underutilised species (NUS).

NUS – also known as orphan crops (Padulosi, 2017) – represent tens of thousands of plant species (Chivenge et al., 2015) that are suitable for human nutrition. NUS are widely claimed to contribute to sustainability and sustainable development (El Bilali et al., 2023b) as they can help addressing environmental degradation, food and nutrition insecurity, water scarcity, poverty and climate change (El Bilali et al., 2023a; Mabhaudhi et al., 2019). NUS contribute to climate-resilient food systems (Mabhaudhi et al., 2019), conservation of agro-biodiversity and agro-ecosystems (Padulosi et al., 2013), reduction of environmental contamination from agriculture (Mabhaudhi et al., 2019), food and nutrition security, especially in developing countries (Mabhaudhi et al., 2019; Padulosi et al., 2013; Ulian et al., 2020) and rural livelihoods (El Bilali et al., 2023a; Kour et al., 2018; Padulosi et al., 2013). Therefore, Mabhaudhi et al. (2016) argue that the promotion of NUS could contribute to the achievement of the Sustainable Development Goals (SDGs). However, it is still difficult to assess the sustainability potential of NUS due to the lack of a specific framework. Following a review on the assessment of the sustainability of NUS, El Bilali et al. (2022) stated "*One of the main results of this analysis is that there is a dearth of quality scholarly documents that deal with the assessment of the sustainability of NUS. This is rather surprising and largely unexpected given the ongoing rhetoric on the enhancement and development of*

NUS and their value chains to address different challenges such as biodiversity loss, climate change, food insecurity and malnutrition, poverty and livelihoods vulnerability" (p. 27). To bridge this gap, the present paper describes a matrix for the assessment of the environmental, social and economic sustainability of NUS developed within the project SUSTLIVES (*SUSTaining and improving local crop patrimony in Burkina Faso and Niger for better LIVes and EcoSystems*).

MATERIAL AND METHODS

This work was carried out within the project SUSTLIVES in Burkina Faso and Niger (SUSTLIVES, 2023). The project focuses on six NUS in each country (SUSTLIVES, 2022):

- Burkina Faso: Sweet potato, Hausa potato/Fabirama, roselle, moringa, amaranth and Bambara groundnut/voandzou.
- Niger: Sweet potato, cassava, roselle, moringa, okra and Bambara groundnut/voandzou.

A first draft of the sustainability assessment matrix was developed based on a review of the literature (El Bilali et al., 2022). After that, the matrix draft was integrated with indicators from other sustainability assessment frameworks and approaches. These were mainly proposed by experts or retrieved from the literature. They include the Sustainability Assessment of Food and Agriculture systems (SAFA) framework (FAO, 2013, 2014), the Sustainability assessment framework developed within the Agriculture and Quality project in Apulia region (South-Eastern Italy) (Capone et al., 2016; El Bilali et al., 2020) and FAOSTAT (FAO, 2022). The selected indicators were required to be specific to the NUS (therefore they should be calculated/assessed for each crop/NUS; indicators regarding for example farms, households or food systems were excluded), easily measurable using available secondary data (indicators whose measurement requires a specific survey, trial or elaborated, demanding data collection were excluded), appropriate for NUS and the contexts of Burkina Faso and Niger, and relevant for the assessment of environmental, economic or social sustainability.

From the data collected, a sustainability evaluation matrix was developed. Then, two workshops were organized in Niamey – Niger (January 2023) and Ouagadougou – Burkina Faso (February 2023) to finalize and validate the matrix (Figure 1). At each workshop, there was first a presentation of the proposed NUS sustainability assessment approach followed by the presentation and discussion of each of the components of the approach (viz. list of indicators and units, scoring system and reference crops). Each workshop was attended by around twenty experts. Experts in the agri-food sector covered the environmental, social and economic dimensions of sustainability.

Figure 1. Workshop in Ouagadougou (Burkina Faso) to validate the sustainability assessment approach.

As for the operationalisation and contextualization of the proposed sustainability assessment approach, in line with Capone et al. (2016) and El Bilali et al. (2020), the developed assessment matrix uses equal weighting for indicators within themes as well as for themes within each sustainability dimension, and arithmetic averages for aggregating the scores of both indicators and themes. A rating and scoring system is developed for each indicator; from 0 (unsustainable) to 10 (very sustainable) with 5 corresponding to the sustainability benchmark value (value of the nearest exemplar crop or reference crop). A NUS is considered sustainable if it has an average score of 5/10 or higher. The calculation of the benchmark of each indicator for each crop is fundamental for sustainability assessment. Indeed, the level of sustainability of a NUS is assessed relative to a reference crop (Dawson et al., 2019). The reference crop is chosen from the main crops in each country within the group of the selected NUS (viz. roots/tubers, vegetables, legumes) and taking into account the product uses.

RESULTS AND DISCUSSION

The validated sustainability assessment matrix – with sustainability dimensions, themes, and indicators set with description and units – is presented in Table 1. It contains 27 indicators divided into themes covering the three dimensions of sustainability: environmental (environmental integrity, agronomic performance and productivity), social (cultural importance and relevance, nutritional quality and diversity, employment, equity and accessibility) and economic (competitiveness, profitability).

Table 1. SUSTLIVES matrix for the assessment of the environmental, social and economic sustainability of NUS.

Dimension	Theme	Indicator	Description and unit
Environmental (Env)	Env1. Environmental integrity	Env1.1 Nitrogen requirement	Quantity of nitrogen needed during a growing season per ha (kg/ha).
		Env1.2 Phosphorus requirement	Quantity of phosphorus needed during a growing season per ha (kg/ha).
		Env1.3 Pesticide requirement	Quantity of fungicides, insecticides and other plant protection products needed during a growing season per ha (kg/ha).
		Env1.4 Water demand	Volume of water needed during a growing season per ha (m^3/ha).
		Env1.5 Crop evapotranspiration	Crop evapotranspiration under standard conditions (ETc) in each country (m^3/ha/day).
		Env1.6 Genetic diversity	Number of known varieties.
		Env1.7 Nitrogen fixation	Amount of nitrogen fixed by the crop during a growing season per ha (kg/ha).
	Env2. Agronomic performance and productivity	Env2.1 Yield	Production during a growing season (t/ha). For crops with staggered harvest, this represents cumulative production, of the main product, over the whole growing season.
		Env2.2 Length of the growing season	Duration of the growing season till the harvest (days). For crops with staggered harvest, this represents the time to the first harvest.
		Env2.3 Growing degree days	Modified growing degree days (GDD), taking into consideration both lower and higher baseline temperatures, till maturity or first harvest (°C).
		Env2.4 Level of tolerance to salinity	Maximum level of soil salinity tolerated by the crop (dS/m).
		Env2.5 Level of tolerance to high temperatures	Maximum temperature tolerated by the crop without significant damages (°C).
		Env2.6 Level of tolerance/resistance to pests and diseases	Number of known key pests, diseases and parasitic plants.
		Env2.7 Seed	Quantity of seeds and planting material

		availability	available in the country on a yearly basis (Tons).
		Env2.8 Seed suitability	Number of domestic varieties, adapted to local conditions, available in the country.
		Env2.9 Seed quality	Number of improved/certified varieties available in the country.
Social (S)	S1. Cultural significance and relevance	S1.1 Number of documented uses	Number of categories of uses documented (human food, technology/tool, medicinal, firewood, animal feed, symbolic/religious/cultural uses, textile, cosmetic).
	S2. Nutritional quality and diversity	S2.1 Content of bioactive and health-promoting compounds	Content (g/kg of fresh produce) of proteins, fibres, vitamins and minerals (potassium, phosphorus, magnesium, calcium and iron).
		S2.2 Protein content	Content of proteins (% of fresh produce).
		S2.3 Duration of fresh produce conservation	Number of days, from harvest, of conservation and storage of produce in ambient conditions without significant deterioration of its quality or its loss.
	S3. Employment	S3.1 Labour requirement	Number of working days per growing season (person-days/ha).
	S4. Equity and fair accessibility	S4.1 Seed access	Seed price during the main planting/sowing period (EUR/ton).
Economic (Econ)	Econ1. Competitiveness	Econ1.1 Price	Producer price of fresh produce, unprocessed products (EUR/ton).
		Econ1.2 Market demand	Consumption in the country (kg/capita/year). Food supply (cf. Food balance sheets of FAO) can be used as a proxy.
		Econ1.3 Production cost	Cost of production for a growing season (EUR/ha).
	Econ2. Profitability	Econ2.1 Gross profit margin	Gross margin for a growing season (EUR/ha). It is the difference between income from product sales and variable costs.
		Econ2.2 Income	Net income for a growing season (EUR/ha). It is the difference between total income and total expenses.

To implement the sustainability assessment matrix, in line with Capone et al. (2016) and El Bilali et al. (2020), the scores of the indicators (not absolute values) are aggregated to obtain an overall score on the performance/sustainability of a NUS (Table 2).

Table 2. Methods of calculation of average scores for sustainability themes and dimensions as well as the overall sustainability score for each NUS.

Score type	**Calculation formula**
Sustainability theme score	*Score Theme I = sum of scores of all indicators of the theme I / number of indicators of the theme I*
Sustainability dimension score	*Score Dimension J = sum of average scores of all themes of the dimension J / number of themes of the dimension J*
NUS overall sustainability score	*Overall sustainability score of NUS N = sum of scores of environmental, social and economic dimensions for NUS N / 3*

To better understand how the scoring system is applied to indicators, an example is provided in Table 3. It distinguishes between 'positive indicators' and 'negative indicators'. For 'positive indicators', there is a positive correlation between the indicator value and its sustainability score i.e. sustainability score increases with indicator value (e.g. yield). Whereas in the case of 'negative indicators', there is a negative correlation between the indicator value and its sustainability score i.e. sustainability score decreases with the increase of the indicator value (e.g. nitrogen requirement).

Table 3. Example of a scoring system applied to a 'positive indicator' and a 'negative indicator'.

Positive indicator			**Negative indicator**		
Indicator value (IV) intervals	Interval central point	Sustainability score	Indicator value (IV) intervals	Interval central point	Sustainabilit y score
IV < IB - 90% IB	-100%	0	IV > IB + 90% IB	+100%	0
IB - 70% IB < IV ≥ IB - 90% IB	-80%	1	IB + 70% IB < IV ≥ IB + 90% IB	+80%	1
IB - 50% IB < IV ≥ IB - 70% IB	-60%	2	IB + 50% IB < IV ≥ IB + 70% IB	+60%	2
IB - 30% IB < IV ≥ IB - 50% IB	-40%	3	IB + 30% IB < IV ≥ IB + 50% IB	+40%	3
IB - 10% IB < IV ≥ IB - 30% IB	-20%	4	IB + 10% IB < IV ≥ IB + 30% IB	+20%	4
Indicator benchmark (IB) +/- 10% IB	0	5	Indicator benchmark (IB) +/- 10% IB	0	5
IB + 10% IB > IV ≤ IB + 30% IB	+20%	6	IB - 10% IB > IV ≤ IB - 30% IB	-20%	6

Positive indicator			**Negative indicator**		
IB + 30% IB > IV ≤ IB + 50% IB	+40%	7	IB - 30% IB > IV ≤ IB - 50% IB	-40%	7
IB + 50% IB > IV ≤ IB+ 70% IB	+60%	8	IB - 50% IB > IV ≤ IB - 70% IB	-60%	8
IB + 70% IB > IV ≤ IB + 90% IB	+80%	9	IB - 70% IB > IV ≤ IB - 90% IB	-80%	9
IV > IB + 90% IB	+100%	10	IV > IB - 90% IB	-100%	10

IB: Indicator benchmark. IV: Indicator value.

A reference crop was identified for each NUS and in each country, and co-validated during the workshops (Table 4).

Table 4. List of the reference crops for NUS selected within SUSTLIVES project.

Country	**NUS selected**	**Reference crop**
Niger	Sweet potato (*Ipomoea batatas*)	Potato
	Cassava (*Manihot esculenta*)	Potato
	Roselle (*Hibiscus sabdariffa*)	Cabbage
	Moringa (*Moringa oleifera*)	Cabbage
	Okra (*Abelmoschus esculentus*)	Pepper
	Bambara groundnut (*Vigna subterranea*)	Cowpea
Burkina Faso	Sweet potato (*Ipomoea batatas*)	Potato
	Fabirama (*Solenostemon rotundifolius*)	Potato
	Roselle (*Hibiscus sabdariffa*)	Cabbage
	Moringa (*Moringa oleifera*)	Cabbage
	Amaranth (*Amaranthus sp.*)	Spinach
	Bambara groundnut (*Vigna subterranea*)	Cowpea

The determination of the reference crop allows calculating the benchmark and developing the scoring scale for each indicator. For example, in the case of potato, the scoring scale for the yield indicator is provided in table 5. The benchmark values are the average potato yields (PY) in Burkina Faso (111832 hg/ha i.e. 11.18 tons/ha) and Niger (311234 hg/ha i.e. 31.12 tons/ha) in 2020 from FAOSTAT. Therefore, for sweet potato, with a yield of 10.5 t/ha, it has a score of 5 in Burkina Faso (viz. 10.06 < PY ≥ 12.30) and 2 in Niger (viz. 9.34 < PY ≥ 15.56).

Table 5. Scoring scales of yield indicator for potato in Burkina Faso and Niger.

Indicator value (IV) intervals	**Burkina Faso – Potato yield (PY) intervals**	**Niger – Potato yield (PY) intervals**	**Sustainability score**
IV < IB - 90% IB	PY < 1.12	PY < 3.11	0
IB - 70% IB < IV ≥ IB - 90% IB	1.12 < PY ≥ 3.35	3.11 < PY ≥ 9.34	1
IB - 50% IB < IV ≥ IB - 70% IB	3.35 < PY ≥ 5.59	9.34 < PY ≥ 15.56	2
IB - 30% IB < IV ≥ IB - 50% IB	5.59 < PY ≥ 7.83	15.56 < PY ≥ 21.78	3
IB - 10% IB < IV ≥ IB - 30% IB	7.83 < PY ≥ 10.06	21.78 < PY ≥ 28.01	4
Indicator benchmark (IB) +/- 10% IB	10.06 < PY ≥ 12.30	28.01< PY ≥ 34.23	5
IB + 10% IB > IV ≤ IB + 30% IB	12.30 > PY ≤ 14.53	34.23 > PY ≤ 40.46	6
IB + 30% IB > IV ≤ IB + 50% IB	14.53 > PY ≤ 16.77	40.46 > PY ≤ 46.68	7
IB + 50% IB > IV ≤ IB+ 70% IB	16.77 > PY ≤ 19.01	46.68 > PY ≤ 52.90	8
IB + 70% IB > IV ≤ IB + 90% IB	19.01 > PY ≤ 21.24	52.90 > PY ≤ 59.13	9
IV > IB + 90% IB	PY > 21.24	PY > 59.13	10
IB	11.18	31.12	

IB: Indicator benchmark. IV: Indicator value.

CONCLUSIONS

To the best of our knowledge, this is the first sustainability assessment approach specifically developed for neglected and underutilized crop species. The approach, co-developed with local actors and stakeholders, provides a tool for efficient, effective and sustainable promotion of NUS in Burkina Faso, Niger and the Sahel and West Africa. The proposed sustainability assessment approach allows not only to objectively assess the environmental, social and economic sustainability of each NUS, and to relate it to that of the main reference crop, but also can be used for the prioritization of NUS to be promoted based on their potential impact. Although the matrix was developed for the NUS selected within SUSTLIVES project (amaranth, fabirama, okra, cassava, moringa, roselle, sweet potato, voandzou), it can be used for other crops and in other countries after a contextualization. In particular, this step implies the identification of the reference crop and the development of the scoring scale. The next step will be the application of the sustainability assessment approach developed on the NUS selected in Burkina Faso (amaranth, fabirama, moringa, roselle, sweet potato, voandzou) and Niger (okra, cassava, moringa, roselle, sweet potato, voandzou) for its final validation.

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BACTERIAL ECTOMICROFLORA OF *VARROA DESTRUCTOR*, ECTOPARASITE OF HONEYBEE, COLLECTED IN THE APIARY OF BOUMERDES (ALGERIA)

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ABSTRACT

Varroa destructor Anderson and Trueman (Acari: Varroadae), previously known as *Varroa jacobsoni*, is an important pest of the honeybee, *Apis mellifera* L. It has been causing severe damage to populations of this species worldwide in recent years. The aim of this work was the isolation and identification of the bacterial ectomicroflora of the *Varroa destructor*, an ectoparasite of the bee (*Apis mellifera* L). Samples of *Varroa* were collected from the location of Boumerdes (situated in northern Algeria) beehive summer debris. The ectoparasitic honeybee *Varroa* was disinfected with 70% ethanol and then it was spread in nutrient agar plates. For the isolation and identification of the bacteria, the macroscopic and microscopic characters were done according to Bergey's manual of systematic Bacteriology. Biochemical characteristics were tested by using API 20E galleries (Biomerieux). The experiments were performed twice. The results of the preliminary study showed that the ectoparasite harbored seven genera of bacteria: *Staphylococcus* sp (3), *Bacillus* sp (2) and *Pseudomonas* sp (2). The colonies of *Staphylococcus* are Gram positive, mobile, coccoid shaped, aero-anaerobic and with a positive catalase. *Bacillus* are Gram-staining-positive rods, mobile, endospore forming, aerobes or facultative anaerobic and can produce catalase and oxidase. *Pseudomonas* bacteria are Gram-negative, oxidase-positive, strict aerobic and non-spore forming.

Key words: *Apis mellifera L, Varroa destructor, bacterial ectomicroflora, beehive summer debris.*

INTRODUCTION

Varroa destructor (Anderson and Trueman, 2000) is the most destructive and important pest in beekeeping wordwide. The mite originates in Asia whose natural host is *Apis cerana* (Le Conte *et al*., 2010). On the new host, *Apis mellifera*

(Hymenoptera: Apidae), *Varroa destructor* induce several damages including the disturbance of the morphological, biochemical and immunological parameters (Weinberg and Madel, 1985; Daly *et al.*, 1988, Marcangeli *et al.*, 1992; Contzen *et al.*, 2004; Yang and Cox-Foster, 2005; Belaïd and Doumandji, 2010; Belaïd *et al.*, 2017). Others effects were caused by the microflora transmitted by the obligatory ectoparasite of the honeybee (*Apis mellifera* L). The mite can transmit American foulbrood (De Rycke *et al.*, 2002).), the sacbrood virus (Bailey, 1991), acute paralysis virus (Ball, 1985, 1988; Ball and Allen, 1988) by inoculating virus particles into the haemolymph of honeybees. The haematophagous is also one of the vectors of fungi (Benoit *et al.*, 2004) and several bacteria (Gliński and Jarosz, 1990 a, 1992). To our knowdelge, there are a few studies have been made about the bacteria of the *Varroa* (Majboroda *et al.*, 2013; Vanikova *et al.*, 2014; Maddaloni and Pascual, 2015). The purpose of the study was to determine the bacteria ectomicroflora of the *Varrao destructor* in *Apis mellifera intermissa* in Boumerdes (Algeria).

MATERIAL AND METHODS

Collection of *Varroa* mites

Samples of adult female mites of *Varroa destructor* naturally fall were obtained from *Apis mellifera intermissa* colonies in summer placed in apiary of the Boumerdes (situated in northern of Algeria) beehive summer debris. The ectoparasitic honeybee *Varroa* was disinfected with 70% ethanol and then it was spreading in nutrient agar plates. The experiments were performed twice.

Isolation and identification of bacteria

For the isolation and identification of the bacteria, the macroscopic and microscopic characters were done according to the Bergey's manual of systematic Bacteriology (Holt *et al.*, 1994). Biochemical characteristics were tested by using API 20E galleries (Biomerieux).

RESULTS AND DISCUSSION

In the work, a total of seven strains were isolated from the ectoparasitic mite *Varroa* destructor collected from different location in Boumerdes (Figure 1, Table 1 and 2).

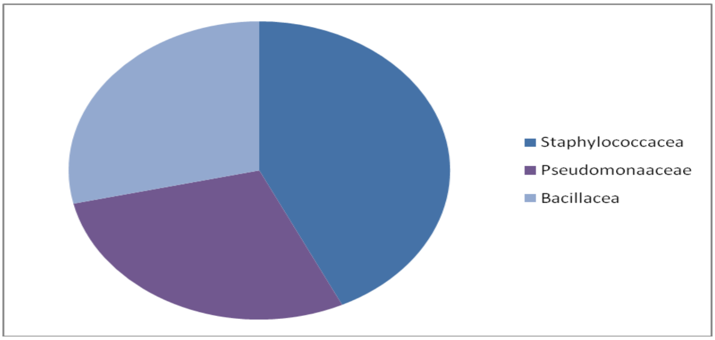

Figure 1: Percent distribution of bacteria isolates

According to the Bergey's manual of systematic Bacteriology (Holt *et al*., 1994), the results showed that the ectoparasite harbored 7 strains of bacteria: 3 belonging of Staphylococaccae (S1, S2 and S7) 42,85%, 2 of Bacillaceae (S3 and S4) and 2 of Pseudomonodaceae (S5 and S6) (Fig 1) 28,57%. The colonies S1, S2 and S7, the members of Staphylococcacea, isolated from the ectoparasite are Gram positive, mobile, coccoid shaped, aero-anaerobic and with a positive catalase. S3 and S4 colonies belonging to Bacillaceae are Gram-staining-positive rods, mobile, endospore forming, aerobes or facultative anaerobic and can produce catalase and oxidase. S5 and S6 are Gram-negative, oxidase-positive, strict aerobic and non-spore forming. The isolates of family Pseudomonadaceae detected during our studies and collected from *Varroa destructor* beehive summer debris were identified as *Pseudomonas* sp (Table 1).

Table 1. Morphological and physiological characteristics of isolated bacteria from *V. destructor*.

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
Shape	c	c	r	r	r	r	c
Gram strains	+	+	+	+	-	-	+
Endospore	-	-	+	+	-	-	-
Respiratory type	a	a	a	an	a	a	an
Oxidase test	-	-	+	+	+	+	-
Catalase test	+	+	+	+	+	+	+
Motility	+	+	+	+	-	-	+

(+): positive test ;(-): negative test; c: cocci; r: rods; a: aerobic; an: anaerobic.

Based on API 20E galleries (Biomerieux), the strains identified as *Bacillus* sp are positive for ortho-nitro-phénol-galactosidase, arginine di-hydroxylase, Ornithine décarboxylase, citrate utilization test, acetoin production, gelatinase but negative for indol production, urease and inositol. In our study, the colonies of *Pseudomonas* sp were positive for ortho-nitro-phénol-galactosidase, arginine di-hydroxylase, ornithine décarboxylase (ODC), citrate utilization test (CIT). The strains were capable of using Glucose, Mannose, Sorbitol, Rhamnose, Saccharose, Melibiose, Amydaline and Arabinose (Table 2).

Table 2. Biochemical characteristics of bacterial isolats

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
ONPG	+	+	+	+	+	+	+
ADH	+	+	+	+	+	+	+
LDC	-	-	-	+	-	+	-
ODC	+	+	+	+	+	+	+
CIT	+	+	+	+	+	+	+
H_2S Production test	-	-	-	-	-	+	-
URE	+	+	-	-	+	+	+

TDA	-	-	-	-	-	-	-
IND	-	-	-	-	-	-	-
VP	+	+	+	+	+	+	+
GEL	+	+	+	+	+	+	+
GL	+	+	+	+	+	+	+
MANE	+	+	+	+	+	+	+
INO	-	-	-	-	-	+	+
SOR	+	+	+	+	+	+	+
RHA	+	+	+	+	+	+	+
SAC	+	+	+	+	+	+	+
MEL	+	+	+	+	+	+	+
AMY	+	+	+	+	+	+	+
ARA	+	+	+	+	+	+	+

(+): positive test ;(-): negative test; Ortho-nitro-phénol-galactosidase (ONPG); Arginine di-Hydroxylase (ADH); Lysin di-Carboxylase (LDC); Ornithine décarboxylase (ODC); Citrate utilization test (CIT); Urease (URE); Tryptophane Désaminase (TDA ; Indol production (IND) ; Acetoin production (VP) ; Gelatinase (GEL) ; Glucose (GL) ; Mannose (MANE); Inositol (INO) ; Sorbitol (SOR) ; Rhamnose (RHA) ; Saccharose (SAC); Melibiose (MEL); Amydaline (AMY); Arabinose (ARA).

A lot papers are published on the subject of the viral transmission such Sacbrood bee virus (Ball, 1999a), *Acute Bee Paralysis Virus* (Faucon *et al.,* 1992; Ball, 1999a; Brodsgaard *et al.*, 2000), *Kashmir Bee Virus* (Ball, 1999 a ; Chen *et al.*, 2004; Nyguyen *et al.*, 2010), virus *Deformed Wing Virus* (Ball, 1999b; Bowen-Walker *et al*.; Tentcheva *et al.,* 2004; Chejanovsky *et al.,* 2010; Nyguyen *et al.*, 2010). But, they have a few numbers of data concerning the fungi and bacteria microflora of the *Varroa*. Hrabak (2003) and Benoit *et al*. (2004) reported the femal adults of the honeybee mite *Varroa destructor* have on their surface and have the potential to disperse fungal spores (conidia) throughout the bee colony (*Aspergillus flavus*, *Penicillium multicolor*, *Penicillium simplicissimum*, *Mucor ramosissimus*, *Mucor indicu*, *Mucor hiemalis* and *Ascosphaera apis)*. According to De Rycke *et al*., (2002), *Varroa destructor* is capable of transporting spores of *Paenibacillus larvae* (the American foulbrood agent) to the surface of its body. Based on the Gallery API 20 E (Bio-Merieux), Belaïd *et al*. (2018) found that the heamolymph worker honeybees parasitized by *Varroa destructor* was contamined by *Bacillus licheniformis, Bacillus mycoide*, *Bacillus coagulans, Brevibacillus chohinensis, Aeromonas hydrophila* and *Pantoa sp.* Glinski and Jarosz (1990 b) reported that using *Serratia marcescens*, microbiological assays have indicated that *Varroa jacobsoni* can harbour this indicator bacterium on its body surface and internally. According to Hubert *et al*. (2017), the location, time of year and degree of infestation by *Varroa* had significant effects on the composition of the bacteriome of honey bee workers. These authors found that varroosis are more important factor than *Nosema ceranae*, *Nosema apis* and *Lotmaria passim* infestation influencing the honey bee bacteriome and contributing to the changes in symbiotic bacterial taxa. In the colonies with high *Varroa* infestation levels (varroosis), the relative abundance of the bacteria *Bartonella apis* and *Lactobacillus apis* decreased. In contrast, an increase in relative abundance was observed for several taxa including *Lactobacillus helsingborgensis, Lactobacillus mellis, Commensalibacter intestini, and Snodgrassella alvi*.

Our preliminary investigations show the presence of several bacterial strains as *Bacillus* sp, *Pseudomonas* sp and *Staphylococcus* sp isolated from the honeybee external body of the parasitic mite *Varroa destructor* collected from Boumerdes beehive summer debris. According to Hrabak (2003), the genus *Staphylococcus albus* and *Enterobacter cloacae* were isolated from the external ectoparasite mite. Bacillacea (*Bacillus sp*) and Micrococcaceae were cited by Tsagou *et al*., (2004). According to Alquisira-Ramírez *et al.*, (2014), fifty-four *Bacillus*-like strains were isolated from dead *Varroa destructor* collected in 24 colonies of bees from seven apiaries. Many bacteria such *Morganella* sp, *Enterococcus* sp, *Pseudomonas* sp, *Rahnella* sp, *Erwinia* sp and *Arsenophonus* sp were identified by Hubert *et al*., (2015). Other bacteria microflora was also recorded by Maddaloni and Pascual (2015) (*Bacillus subtilis*, *Burkholderia*, *Pseudomonas syringae*, *Pantoa agglomerans*, *Pantoa vagans*, *Paenibacillus* wynnii, *Staphylococcus caprae*, *Bifidobacterium asteroids*, *Staphylococcus caprae*, *Micrococcus luteus* etc. and by Vanikova *et al*. (2015) (*Microbacteruim* sp and *Bacillus* sp). The most abundant bacteria in *Varroa* mites belonged to the family *Enterobacteriaceae*, especially the genera *Arsenophonus*, *Enterobacter* and *Proteus*. axon-specific *Enterobacteriaceae* and *Arsenophonus* probes also confirmed their localization in the cecum of *Varroa (*Pakwan *et al*., 2018). The external body of *Varroa destructor* is not only place where microorganisms could reside. The salivary glands and gut are also colonized by the microflora (Ball, 1997).

CONCLUSION

The preliminary study showed that the ectoparasitic mite, *Varroa destructor*, harbored 7 genera of bacteria: 3 belonging of Staphylococaccae, 2 of Bacillaceae and 2 of Pseudomonodaceae. The bacteria associated with the mite can play an important role in the phenomenon called Colony Collapse Disorder.

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REBOUND EFFECT OF IRRIGATION SUBSIDY PROGRAMS IN ALGERIA

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ABSTRACT

Irrigated agriculture in Algeria plays an essential role in the national economy and food security historically facing significant water scarcity challenges. This problem has intensified over time, with an average annual rainfall of 89 mm and a weighted rainfall index of 241.50 mm for agricultural land. Furthermore, with an average water endowment of 380 m^3 per person per year in 2021, Algeria is classified as a country with limited water resources. Moreover, Algeria has implemented irrigation subsidy programs to boost the agricultural irrigation sector since 2000. However, it is of crucial importance to assess the possible rebound effect of these subsidies, as they may result in an increase in overall water consumption despite efficiency increases made by enhanced irrigation techniques. The aim of this research is to investigate the impact of irrigation subsidies on water consumption in Algeria. Data from 2001 to 2020 on irrigation sector in Algeria show comparable trends to China, India, Morocco, Tunisia, Australia, the United States, and Spain, where water consumption quantities have mainly increased due to subsidy programs. As a result, the already-stressed water resources are strained even further. These findings highlight the critical importance of prudent and sustainable water resource management for the public policy in the context of irrigated agriculture in Algeria.

Keywords: *Irrigation, rebound effect, subsidy programs, Algeria.*

INTRODUCTION

The aim of this study is to assess the rebound effect at aggregate level in irrigated Algerian agriculture caused by irrigation subsidy programs. Algeria is facing an increasing scarcity of water resources, with an average rainfall of 89 mm per year (FAO, 2015) and a water endowment of less than 400 m^3 per person per year in 2021. Irrigated agriculture accounts for a significant share of the agricultural land, utilizing over 70% of the mobilized water resources. To address these challenges, Algeria has implemented agricultural development programs focused on the adoption of water-efficient irrigation techniques such as drip irrigation and sprinkler systems. However, it seems crucial to assess the effectiveness of these measures by drawing insights from international experiences. It is necessary to determine whether promoting more efficient irrigation techniques is a wise choice to enhance water conservation in Algeria, considering lessons learned from countries facing similar conditions. Strategic thinking is required to assess the continued implementation or potential revision of these techniques based on the country's specific needs.

MATERIAL AND METHODS

To address our research problem, we collected data on water consumption for different irrigated crops, irrigated areas utilizing both surface and groundwater sources, as well as areas equipped with water-saving technologies in Algeria. These data were obtained from the Algerian Ministry of Agriculture and Rural Development and the Ministry of Water Resources and the Environment. Furthermore, we conducted a literature review to enhance the work by incorporating existing research and studies conducted in other countries, providing insights into the effects of irrigation modernization. Our study also highlights the irrigation paradox in the Algerian context, emphasizing the challenges and contradictions faced by the country in managing water resources for agriculture.

Subsiding Irrigation Water Efficiency: A Brief Literature Review

Irrigation has always been recognized as the primary consumer of water in terms of evapotranspiration (ET) and a significant source of water wastage, making it a target for measures aimed at conserving this precious resource (Falkenmark and Molden, 2008). To address water scarcity, many governments subsidize and encourage the use of efficient irrigation technologies (EIT) as a means to reduce water consumption and reallocate resources to other users (Grafton et al., 2018; Scott et al., 2014). Investments in these technologies, widely used to achieve water savings, have become a key lever in many countries (Kendy et al., 2004; Batchelor et al., 2014; Molle & Tanouti, 2017; Frija et al., 2014; Ferchichi et al., 2018; Amichi et al., 2015; Grafton et al., 2018; Belaidi et al., 2022; Belaidi, 2013; Oulmane et al., 2020; Wheeler et al., 2018; Gordon et al., 2020; Berbel et al., 2019; Sampedro Sanchez, 2020).

These water-saving policies in agriculture, implemented in several basins worldwide, such as China, India, Morocco, Tunisia, Algeria, Australia, the United States, and Spain, have two main objectives: firstly, to save water for other uses or environmental purposes, and secondly, to increase agricultural income and water productivity by improving yields and promoting diversification towards higher value crops (Perry & Steduto, 2017). However, the results of these programs have not always met expectations and, in some cases, have even contradicted the objectives of preserving water resources (Perry & Steduto, 2017). Analyses conducted on cases such as India, China, Morocco, Australia, and Spain converge to a similar conclusion: while the modernization of irrigation systems has brought benefits in terms of agricultural production and crop diversification, the total water consumption has generally increased, thus creating increased pressure on available basin-scale resources. This phenomenon, known as the "irrigation efficiency paradox" (Grafton et al., 2018).

Some studies have indicated that the efficiency of drip irrigation can be lower than that of surface irrigation due to issues such as inadequate maintenance and poor irrigation practices (Wolf et al., 1995; Benouniche et al., 2014). Furthermore, instances of over-irrigation have been observed in several irrigation systems, attributed to the aim of increasing agricultural production and issues with the design and maintenance of the irrigation network (Benouniche et al., 2014). It is worth noting that enhancing irrigation efficiency does not always guarantee water savings, as it may require increased technical expertise from users (Wittling & Molle, 2017).

The adoption of Water-Saving Irrigation Techniques (WSITs) in the Gujarat and Maharashtra regions has led to a significant change in crop rotation (Namara et al., 2005). In the irrigated area of Zaouiet Jedidi, the adoption of drip irrigation by farmers was motivated not only by the desire to save water but also by other factors such as agricultural intensification and labor reduction, as highlighted by Ferchichi et al. (2018). In the Cànyoles watershed, research by Sese-Minguez et al. (2017) revealed that the adoption of drip irrigation was associated with intensified agricultural practices and a shift towards arboriculture. Similar observations have been made in other irrigation systems in Spain, as noted by studies conducted by González-Cebollada (2015), Sese-Minguez et al. (2017), and Berbel et al. (2014), which observed modifications in planting density and crop patterns. The adoption of WSIT offers farmers, particularly those reliant on well or borehole capacity for water supply, the opportunity to use reduced water quantities applied per hectare to expand irrigated areas.

However, it should be noted that transitioning to drip irrigation can sometimes result in an overall increase in water demand compared to the initial demand, as seen in the case of the Kairouan plain in Tunisia, where there has been an expansion of irrigated areas for the most profitable crops (Feuillette, 2001). This phenomenon has been widely observed in several countries, including Spain, Jordan, Tunisia, India, Pakistan, Israel, China, and the United States (Molle, 2017). The studies by Pfeiffer & Lin (2014), Ward & Pulido-Velazquez (2008), as well as Li & Zhao (2018), demonstrate that promoting more efficient irrigation technologies does not guarantee the conservation of groundwater resources.

In Morocco, the major irrigated plains and coastal aquifers are facing water deficits, indicating overexploitation of water resources (Molle & Tanouti, 2017). Irrigated agriculture, supported by ambitious agricultural policies, intensifies the use of groundwater, contributing to the overexploitation of 50% of the aquifers in Morocco (Kuper et al., 2016). Therefore, the question of whether EITs can reduce pressures on water resources remains relevant. There is controversy surrounding the water-saving attribute of EITs: are they truly effective in saving water? How is it that total irrigation water consumption increases despite the use of more efficient technologies? However, water management in agriculture involves multiple scales, ranging from individual fields and farms to irrigation perimeters, basins, nations.

Understanding and assessing the impact of EITs on water consumption, therefore, requires an integrated approach that takes into account these different scales and the complex interactions among environmental factors, agricultural practices, and water management policies.

Irrigation Subsidy Program in Algeria: An Overview

In Algeria, the subsidy program aims to support various sectors of the economy, including agriculture, to promote development and growth. This program involves allocating financial funds or providing incentives to individuals in order to facilitate their activities. Indeed, several agricultural development plans have been implemented, covering the periods from 2000 to 2019 and from 2020 to 2024. The National Agricultural Development Program (known as PNDA) stood out for its considerable ambition, with a funding of 40 billion DZD in the year 2000. Between 2001 and 2004, the allocated amount for this plan, which was later replaced by the National Agricultural and Rural Development Plan (known as PNDAR) in 2002, amounted to 525 billion dinars (Imache et al., 2011; Salhi & Bedrani, 2011).

In August 2008, the Agricultural Orientation Law[1] (Law No. 08-16) reaffirmed the Agricultural and Rural Renewal Policy by strengthening the strategic option of widespread and extensive use of water-saving systems . The budget specifically allocated to the rural development component for the period 2005-2010 was estimated at around 2.6 billion euros (Kherbache, 2021). According to Benaissa (2022), the support provided during the period 2000-2010 by sector (FNDIA, FNRDA), irrigation was the largest beneficiary with 37.65%, adding up to 242 billion DZD. The MADR presented the water-saving program in the hydro-agricultural sector (2010-2014), focusing on the development of water-saving systems covering an area of 900,000 hectares.

The program implemented between 2014 and 2019 aimed to irrigate 2.14 million hectares by the end of 2019. An investment of approximately 3 billion USD was planned to support this initiative. The total amount planned for the support of water-saving irrigation equipment acquisition alone was almost 948 million USD for an area (subsidized) to be equipped of 637,000 hectares. The 2000-2024 program aims to generalize Water-Saving Irrigation Techniques (WSIT) on a total of 150,000 hectares, with a planned conversion of 30,000 hectares per year over this period (MADR, 2020). Each newly irrigated hectare must be equipped with water-saving systems, including with the contribution of state support.

The current state support covers 50% of the cost of irrigation equipment for individual acquisition and 60% for collective acquisition (Decision no. 943 MADR, 2014). Supported equipment must now pass the crash test of the specialized laboratory of INSID to verify their real efficiency, in accordance with the efficiency rates announced by suppliers. In case of non-compliance, public subsidy will be refused to preserve public funds.

[1] Journal officiel Algérien no 46 du 10 Août 2008, p. 3.

RESULTS AND DISCUSSION

5.1. Evolution of irrigated areas from 2000 to 2018

In Algeria, the irrigated area encompasses two distinct categories characterized by both the size of the developments and the management approach: *Grands Perimeters of Irrigation* (GPI): These are large-scale irrigation projects with a contiguous area greater than 500 hectares. They are under the authority of the National Office of Irrigation (ONID) and primarily utilize surface water from reservoirs, supplemented by deep wells. The management and operation of these projects are carried out by the ONID. *Small and Medium Hydraulic* (PMH): This refers to small and medium-scale private irrigation, directly managed by individual farmers or, in some cases, by associations. The irrigated areas within this category vary in size and predominantly rely on shallow wells or small-scale pumping systems.

The PMH represents a significant portion of the irrigated land in Algeria. In fact, the irrigated area has experienced a substantial increase, growing from 310,000 hectares in 2000 to 1,259,420.95 hectares in 2018. As for the GPI, prior to 2005, their total area did not exceed 50,000 hectares. In 2000, the area was around 40,000 hectares, reaching a maximum of 47,585 hectares in 2004. However, during the period from 2005 to 2018, the irrigated area surpassed an average of 80,000 hectares. This data indicates that the PMH plays a significant role in the overall expansion of the irrigated area in Algeria, with notable growth observed over the years. Furthermore, the GPI also experienced an increase in irrigated land, particularly after 2005. The total irrigated area in Algeria has experienced a significant increase over the years, growing from 350,000 hectares in 2001 to 1,330,670 hectares in 2018 (as shown in Figure 1). Irrigated areas in GPI and PMH reached 1,424,262 hectares in 2020, with 95,259 hectares in large hydraulics and 1,329,003 hectares in small and medium hydraulics. As a result, this expansion highlights the growing importance of irrigation in the country's agricultural sector. These results generally show a rebound effect in irrigated perimeters and small and medium-scale hydraulics, since there has been and will continue to be potential for increasing irrigated areas.

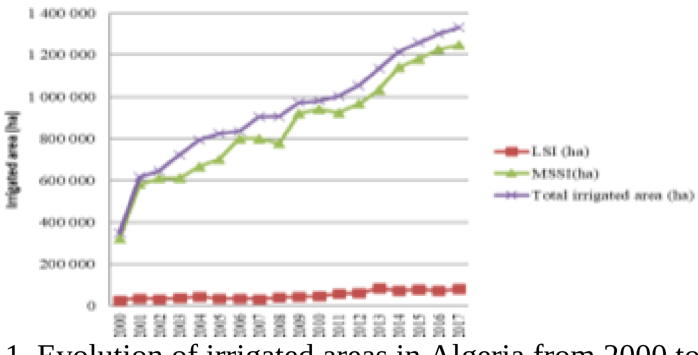


Figure 1. Evolution of irrigated areas in Algeria from 2000 to 2018

5.2. The irrigated areas by irrigation method

In Algeria, various agricultural development plans have been launched with a focus on the widespread adoption of Water-Saving Irrigation Techniques (WSITs). These plans include the conversion of existing gravity irrigation systems to localized and sprinkler systems. Financial support for irrigation development and water conservation promotion ranges from 40% to 60%, depending on the usage and regions. A significant evolution of WSITs has been observed in Algeria between 2000 and 2020.

Between 2001 and 2018, water-saving systems have undergone a considerable evolution thanks to the implemented support programs. There has been a significant increase in the irrigated area using drip irrigation technique, from 5,000 hectares in 2001 to 312,788 hectares in 2018. Similarly, the irrigated area using sprinkler irrigation method increased from 70,000 hectares to 444,706 hectares during the same period. As for the gravity irrigation method, the irrigated area expanded from 275,000 hectares in 2001 to 573,175 hectares in 2018 (Figure 1). The distribution of irrigated areas by technique shows that gravity irrigation covers 43.07% of the total area, sprinkler irrigation occupies 33.42%, and localized irrigation (drip irrigation) represents 23.51% in 2018. Approximately 62% of the total irrigated area is covered by water-saving systems, according to MADR (2020) report.

Since the implementation of irrigation support plans, there has been a trend towards stabilization of the irrigated area using the gravity method. These figures demonstrate the notable progress in the adoption of Water-Saving Irrigation Techniques (WSITs) in the country, showcasing a commitment towards more efficient water use in the agricultural sector.

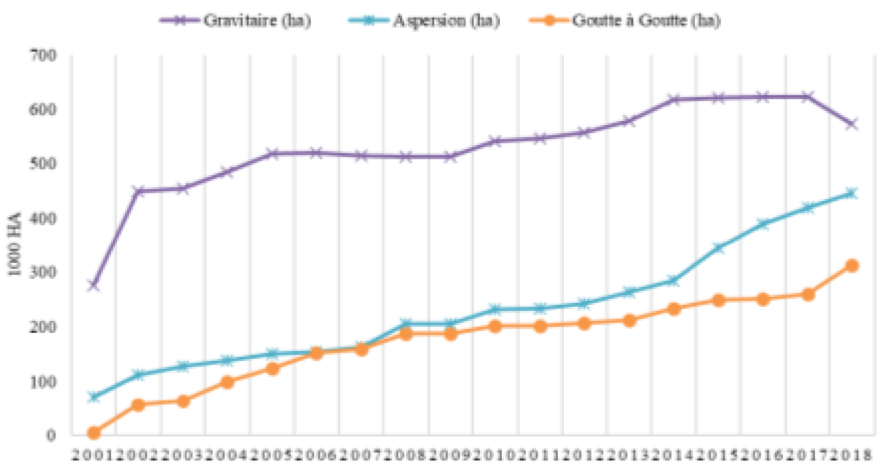


Figure 2. Irrigated areas by irrigation method from 2001-2018 (MADR, 2019)

Concerning the evolution of irrigated areas by irrigation method for large-scale irrigation (GPI) and small and medium-scale irrigation (PMH), Figure 2 indicates that traditional irrigation remains the most widely practiced method in the public perimeters managed by ONID, accounting for 42% of the total irrigated area for the 2020 irrigation season. However, this method has been declining since 2017. Sprinkler irrigation, on the other hand, represents 39% of the total irrigated area for the same irrigation season, showing an increase compared to 2017. Finally, drip irrigation covers 19% of the total irrigated area for the 20012020 irrigation campaign.

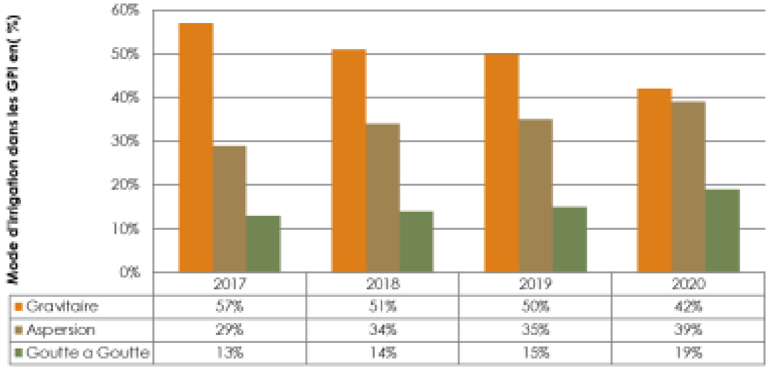


Figure 2. Irrigated areas in GPI by irrigation method in percentages 2017-2020 (ONID, 2021)

Figure 3 indicates that the irrigated areas have increased in recent years, and the installation of water-saving irrigation systems has followed the same trend. In 2003, out of 612,289 hectares irrigated, localized irrigation accounted for only 11.6%. However, by 2006, it had increased to 21.5%, and in 2011, it represented 20.5% with 189,686 hectares irrigated through drip irrigation. This indicates a conversion of existing gravity systems to localized irrigation, while sprinkler irrigation fluctuates between 18% and 19% of the total overall.

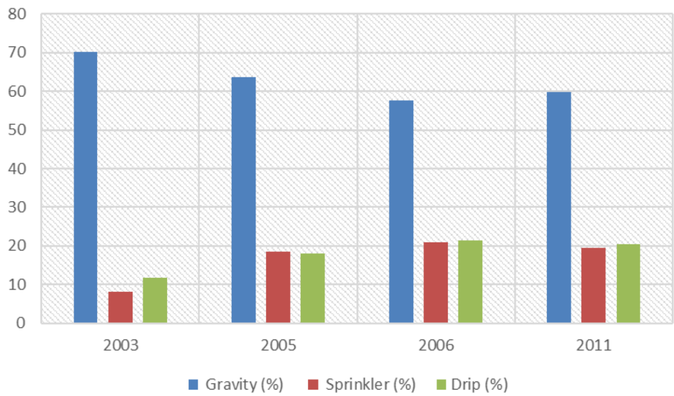
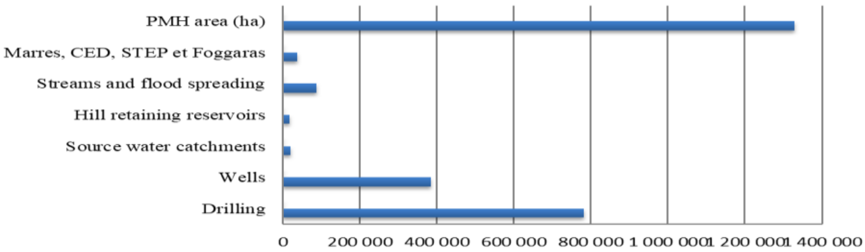


Figure 3. Evolution of PMH irrigated areas by irrigation method

On the PMH areas by origin of water used, the data (shown in Figure 3) indeed confirms a significant development of thousands of wells and boreholes, whose exploitation often causes irreversible harm to groundwater aquifers. Despite the scarcity of water resources, the dynamism of this sector seems to be linked to the autonomy it provides (i.e. owning individual wells and boreholes) to farmers in managing water resources. The source of water used for PMH varies from one region to another based on climatic conditions and water availability. At the national level, PMH continues to rely on groundwater for 90% of its water needs (with a proportion of 100% in the southern regions). This could pose long-term risks to groundwater aquifers and fossil aquifers, especially considering the increasing number of wells being drilled.



	Drilling	Wells	Source water catchments	Hill retaining reservoirs	Streams and flood spreading	Marres, CED, STEP et Foggaras	PMH area (ha)
Percentage(%)	58,92	28,89	1,49	1,36	6,53	2,82	100
Area (ha)	782 986	383 895	19 778	18 023	86 778	37 543	1 329 003

Figure 4: Irrigation of Small-Scale Irrigation (PMH) areas by different sources of irrigation in 2020.

Algeria heavily relies on its groundwater resources. Indeed, alarming overexploitation has been observed in more than half of the Algerian aquifers (Kuper et al., 2016). The number of agricultural wells and boreholes has seen a significant increase, rising from 120,000 in 2000 to 238,340 in 2016. According to the 2019 National Water Plan (PNE), the PMH utilizes a total of 79,508 drilling and 15,5719 wells (as shown in Figure 5).

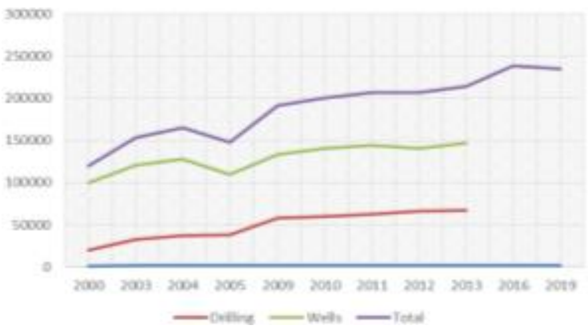


Figure 5: Evolution of number of boreholes and wells from 2000 to 2019

These data globally indicate the rebound effect on water withdrawals, with irrigators who received subsidies and authorizations[2] for irrigation infrastructure significantly increasing their water usage. However, the specific hydrological impact of this rebound effect on water withdrawals at the watershed scale remains unknown.Several territories in Algeria are facing water imbalance issues. The situation of the Macta aquifer is a notable example (Kherbache, 2021), as well as the Chéliff basin (El Meddahi et al., 2014; Amichi, 2013), the plateau of Mostaganem (AGIRE, 2016; Haouichine, 2012; SOGREAH, 2009), the Mitidja region (Imache et al., 2011), and the Biskra region (Amichi et al., 2018; Daoudi et al., 2017).
Table 1 displays the planting densities and number of trees per hectare in Algeria. It is evident that the planting densities under drip irrigation are higher compared to those under gravity irrigation. Consequently, densification and intensification could partially explain these increases in overall water consumption.

Table 1: Planting Density and Number of Trees per Hectare by Irrigation Method in Algeria

Crops	Gravity		Drip irrigation	
	Density	No. of trees per hectare	Density	No. of trees per hectare
Clementine/ Mandarin Orange, lemon	6 x 4	417	4 x 2	1250
	5 x 5	400	6 x 2	833
	6 x 5	333	4 x 4	625
	6 x 6	278	6 x 3	555
	4 x 7	357	4 x 2,5	1000
	6 x 3	555	4 x 4	625
Arboriculture: Apple, Pear, Peach, Apricot	4 x 7	357	4x2	1250
	6 x 6	278	4x2.5	1000
	5 x 7	286	4x3	833
	4 x 6	417	5 x 1.5	1333
	5 x 5	400	4 x 4	625
	5 x 4	500	4 x 3.5	714

Turning now to the evolution of total water consumption in the irrigation sector in Algeria. The government has promoted the use of new technologies and water-saving technologies for agricultural purposes, aiming to improve water efficiency in the sector. In 2018, approximately 70% of the mobilized water potential, which amounted to 8 billion m3, was allocated to agriculture, compared to less than 40% in 2000, which amounted to 1.8 billion m^3 (Pnud, 2020). However, the introduction of water-saving equipment at the plot level has not resulted in overall water savings at the national level. On the contrary, the total water consumption by the irrigated sector has increased (as shown in Figure 6).

[2] According to the Agence Nationale des Ressources Hydrauliques (ANRH), more than 20,000 permits for drilling and wells have been allocated to farmers for the year 2021.

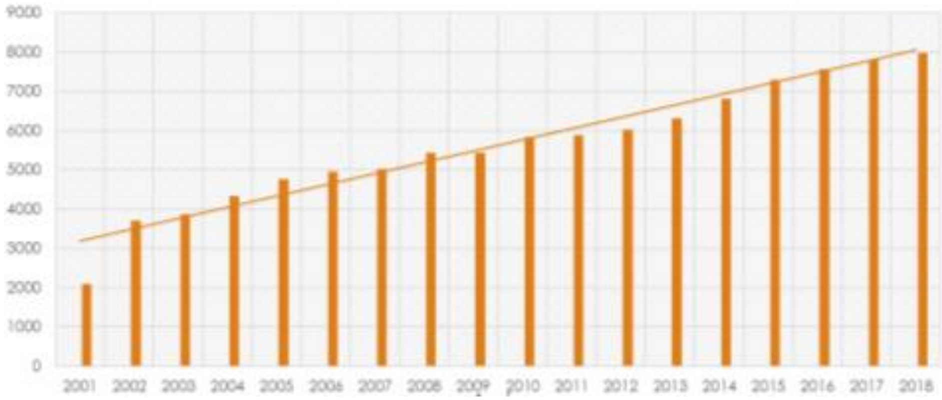


Figure 6. Evolution of irrigation water consumption for the period 2001-2018 (MADR ,2019)

The overall water consumption by irrigated areas, across all irrigation techniques, has risen from 2.10 billion m^3 in 2000/2001 to over 7.98 billion m^3 in 2017/2018. Nevertheless, at the farm level, the adoption of water-saving technologies has led to increased profitability due to significant additional benefits. The saved water has been utilized to intensify the cropping system. However, it is worth noting that the major objective of reducing overall water consumption has not been achieved. Instead, the focus has been on reducing water consumption per hectare in order to expand the cultivated area.

CONCLUSIONS

The aims is to demonstrate that despite irrigation subsidy programs, the overall water consumption in Algeria continues to increase in the irrigated sector (rebound effect), as well as the extensive reliance on groundwater, leading to the worsening overexploitation of aquifers. The Algerian government promotes the adoption of more efficient irrigation technologies to achieve water savings, but this claim is questioned in the literature. Using efficient techniques should, therefore, make it possible to achieve similar agricultural yields to those initially obtained using a smaller volume of water. However, a more efficient irrigation system may paradoxically encourage farmers to increase their water usage. This phenomenon is known as the rebound effect. The purported water savings are based on measurements in experimental stations and do not reflect actual efficiency on farms. In many cases, actual efficiency may be similar to gravity irrigation due to equipment issues and inadequate irrigation practices. Farmers can adjust their behavior by opting for more profitable and water-intensive crops or by irrigating previously non-irrigated land. This can offset the efficiency gains achieved by irrigation technologies.

Even when water savings are achieved at the plot level, it is common to observe an increase in water demand at other scales, such as the farm, regional, or basin level. The adoption of efficient irrigation technologies often encourages farmers to expand irrigated areas or cultivate water-intensive crops. The lack of contracts and monitoring between the government and farmers for the adoption of efficient irrigation technologies, as well as limited subsidies for equipment installations, affects the expansion of irrigated areas. By prioritizing increased production over water savings, the government neglects the consequences on groundwater resources, which are an essential source of irrigation in Algeria. Water-saving policies need to be reassessed, taking into account farmers' behavior, crop choices, and the expansion of irrigated areas. This requires a comprehensive and integrated approach to effectively assess water consumption in agriculture.

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ELEMENTAL COMPOSITION AND STRUCTURE OF HUMIC ACIDS IN SOD-PODZOLIC ARABLE SOIL AND ITS VIRGIN ANALOGUES IN LONG-TERM STATIONARY EXPERIMENT

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ABSTRACT

The effect of crop rotations with different saturation by legumes (0; 28.6; 42.9 percent of legumes) and continuous barley on the elemental composition and structure of humic acids in sod-podzolic heavy loamy soil *(Eutric Albic Retisols (Abruptic, Loamic, Cutanic)* was studied in long-term (since 1977) stationary experiment in Perm Region. The arable soil was compared with virgin analogues (under mixed forest, poaceous-forb meadow) and neglected field. The carbon content in humic acids of arable and virgin sod-podzolic soil was 28.3-33.5, hydrogen 40.1-47.5, oxygen 19.6-27.9, nitrogen 2.1-2.5 and sulfur 0.1-0.2 percent. The differences in humic acids composition of arable and virgin soils result from the qualitative composition of the organic soil material, its amount and the intensity of anthropogenic impact. The saturation of field crop rotation with legumes up to 42.9 percent caused the raising the aliphatic groups share in the structure of soil humic acids (the maximum H / C ratio 1.67 was noted). Using five-field grain rotation promoted the increasing of stable aromatic structures and nitrogen decline in humic acids (the minimum H / C ratio 1.20 and maximum C / N ratio 16.2 was noted). The highest content of oxygen-containing groups and high degree of humic acid oxidation (W = + 0.53), were found in humic acids of neglected field soil (C / O - 0.95). That may indicate the most favorable conditions for the humification of organic matter. According to the results of Fourier IR spectroscopy, the most branched structure, consisting from large amount of individual monomers of various molecular weights with a large number of aliphatic groups, had humic acids in soil under crop rotation with high legumes saturation and soil under mixed forest. Aromatic structures were more intensely expressed in humic acids spectra of the soil under conventional crop rotation and continuous barley.

Key words: *humic acids, elemental analysis, IR spectroscopy, crop rotation, neglected field, continuous barley.*

INTRODUCTION

In the second half of the 20th century, it was believed that the predominant part of soil organic matter (SOM) was represented by humic substances - stable high molecular polymers with heterocyclic compounds enriched by nitrogen, with aromatic core and extensive aliphatic periphery, which give great variety and specificity of its properties and functions. The ratio between himic (HA) and fulvic acids (FA) reflects the SOM quality (Kholodov. et al., 2011; Semenov et al., 2013; Ivanov et al., 2017) These views contradict with modern studies carried out by Russian and foreign scientists at the beginning of the 21st century. New information about genesis, composition and structure of humic substances was provided by instrumental methods of analyses (Fourier IR spectroscopy, , mass spectroscopy, nuclear magnetic resonance, X-ray structural analysis, electronic microscopy, etc.) (Wershaw, 2004; Kleber, Johnson, 2010; Xu et al., 2017).

Three-layer SOM model has been proposed, with biomolecules of amphiphilic organic matter (OM) self-linkage into supramolecular aggregates ordered by non-valent interactions on chemically active surfaces of mineral particles (Kleber et al., 2007; Baveye, Wander, 2019; Kholodov et al., 2020). The multilayer model explains the stability and biodegradability inherent to SOM, much better than the polymer concept (Piccolo, 2002; Olk et al., 2019).

According to the opinion of International Humic Substances Society (IHSS) and the American Soil Science Society, the main criterion for determining humic substances today is still the solubility in alkalis (Kleber, Lehmann, 2019). The different solubility of humic substances in acid- alkaline media is the basis for their division into humic acids, fulvic acids and non-extractable residue (humin). Among humic substances, humic acids (HA) are of particular importance, since they possess high functional activity, determine the specificity of the water, physical, chemical and thermal properties of the soil. Their content and structure depend on the conditions of soil formation and change under anthropogenic impact on the soil (Orlov, 1990; Kholodov et al., 2011).

The aim of given research is to reveal the influence of various land use methods on the elemental content and structure of humic acids in arable soil and to compare it with virgin analogues.

MATERIAL AND METHODS

The experimental work was fulfilled in 2019–2021 in long-term stationary field experiment (founded in 1977) on the experimental farm of Perm Agricultural Research Institute. The experimental plots located on sod-podsolic heavy loam soil. Experimental scheme: 1. continuous barley; 2. five-course grain field rotation (barley, winter rye, spring wheat, barley, oat; with 0 percent of legumes); 3. seven-course conventional field rotation (manured bare fallow, winter rye, spring wheat as cover crop for red clover, first year clover, second year clover, barley, oat; with 28.6 percent of legumes) 4. seven-course field rotation (green manure fallow (clover), winter rye, spring wheat as cover crop for red clover, first year clover, second year clover, barley, oat as cover crop for red clover; with 42.9% of legumes) 5. neglected field (no tillage since 1978). There were no mineral fertilizers application in presented variants. The soil had the following agrochemical characteristics by the time of experiment foundation: pH_{KCl} 5.2 - 5.3; hydrolytic acidity 2.1 - 2.3 cmol (eq)/kg, the the sum of exchange bases (S) was 14.0-15.5 cmol (eq) / kg, the content of organic carbon according to Tyurin was 1.10-1.12%, available phosphorus P_2O_5 225-240 mg/kg, exchange potassium K_2O - 196-204 mg / kg (according to Kirsanov). Soil samples for research were taken in the fall of 2018 from the 0-20 cm layer in two non-contiguous replications.

To identify the processes of organic matter transformation, arable soils were compared with their virgin analogs: under mixed-deciduous forest with rich grass cover and poaceous-forb meadow. The following tree species were presented in the forest: coniferous - spruce (*Pícea ábies*), fir (*Ábies sibírica*), pine-tree (*Pinus sylvestris)*, deciduous - birch (*Betula pendula*), aspen (*Populus tremula*) and maple (*Acer platanoides*). Mountain ash (*Sorbus aucuparia*), linden (*Tilia cordata*), alder (*Álnus incána*), bird cherry, (*Prunus padus*) etc. formed well developed forest undergrowth. Oxalis, oxalis-ferns and forb-poaceous-ferns plant communities prevailed in the ground cover. The thickness of the forest litter under the forest canopy was about 3 cm. The species composition of the natural poaceous-forb meadow: poaceous grasses – 62.0 percent; legumes – 13.5 percent; forbs – 24.5percent. Soil samples under the mixed forest were taken from the layer 3-20 cm. Preparations of humic acids were separated according to the classical method of the Russian School of Soil Science, which differs from the recommendations of the International Humic Substances Society (IHSS) (Swift, 1996). The soil was extracted with alkali at least three times in ordinary air, then combined extract is analyzed. The elemental composition of humic acids was determined on a CHN - elemental analyzer after Perkin - Elmer company (USA), the amount of oxygen was calculated by difference (all calculations are given for ash free preparations); IR absorption spectra were recorded on a VERTEX-80v Fourier spectrometer (Bruker company, Germany) in the range 4000-400 cm-1 at a spectral resolution of 2 cm-1. The spectra were processed using the OPUS software package.

The research work was carried out in the IV agroclimatic district of Perm Region, located in the geographical subzone of the southern taiga and mixed coniferous-deciduous forests (Agro-climatic resources…,1979). In accordance with the soil-ecological zoning, the territory of Perm Region belongs to the Vyatka-Kama soil province. The climate is temperate continental with cold, long, snowy winters and warm short summers. The sum of the average daily temperatures above 10°C is 1700-1900. The duration of the active growing season with temperatures above 10°C is on average 115 days, with temperatures above 15°C – 60 days. The district belongs to the zone of sufficient moisture: hydrothermal coefficient (HTC) –1.4, annual precipitation sum is 470-500 mm, evaporation from the soil surface is about 340 mm. The number of days with snow cover averages 176 (Eremchenko et al., 2016).

RESULTS AND DISCUSSION

The main peculiars of the studied soils is the low carbon content in the upper layers: under mixed forest -1.57%, poaceous -forb meadow -1.25, in arable soil - 1.01-1.47% (depending on the experiment treatment), high acidity (pH 4.2; 4.8 and 4.8-5.5 respectively) (Table 1). Nitrogen content was medium and high, total C:N ratio was 8.3-9.4, under mixed forest it was high – 5.9, under the meadow - average - 8.4 (according the scale by Orlov, Grishina, 1981).

Table 1. Agrochemical properties of sod-podzolic soil and ecophysiological indicators of microflora condition under different land use

Type of land using	C_{org}, %	pH_{KCl}	S	Ha	Ca	Mg	P_2O_5,	K_2O	N_{tot}	Cmic	SIR
			cmol (eq) / kg,				mg/kg			μg/g	μg/g C-CO_2/g hour
Continuous barley,	1.01	5.1	22.5	3.1	18.6	2.0	440	212	1078	413	10.3
Grain crop rotation (0% of legumes)	1.07	5.2	20.1	3.2	17.9	3.6	420	188	1295	189	5.2
Conventional crop rotation 28.6 % of legumes)	1.47	5.5	21.5	3.0	17.2	3.2	537	322	1729	316	8.3
Crop rotation (42.9 % of legumes)	1.21	4.9	21.7	3.5	18.2	2.9	314	219	1428	257	5.1
Neglected field	1.36	4.8	19.8	4.8	17.2	2.8	473	247	1554	440	10.9
Mixed forest	1.57	4.2	20.0	6.4	12.0	3.2	168	177	2660	1236	30.9
Poaceous-forb meadow	1.25	4.8	21.2	2.2	13.9	2.5	290	175	1490	571	14.3
LSD_{05}	0.19	0.2	$F_ф<F_т$	0.4	1.2	$F_ф<F_т$	58	34	111	128	1.2

The noted differences in the carbon content in the experiment variants are characteristic of sod-podzolic soil, poor in humus and COM. For more fertile soils, the difference may not be so noticeable. So in research of Seremešić et al (2021), on chernozem soil the average content of SOM was similar for organic and conventional management practices – 3.09% and 3.08%, respectively. Thus, the additional research is required for each type of soil and region with different natural conditions. The studied soils, in accordance with the approximate scale of soil enrichment with microflora, correspond to the gradations of “very poor” and “poor” (Zvyagintsev et al., 2005) The virgin soil was characterized by the minimum content of all microorganisms types (Zavyalova et al., 2020; Zavyalova et al.,2021). The carbon content of microbial biomass (Cmic) varied from 189 in grain rotation to 1236 μg/g soil under mixed forest. The maximum value of substrate-induced respiration (SIR) was recorded in the study of soil under mixed forest - 30.9 μg C-CO2/g hour. To obtain information about the structure of supramolecular aggregates, the presence of basic constitutional elements in their structural fragments, and the transformation direction of organic matter under the influence of natural and anthropogenic factors, we used the method of elemental analysis in the study of humic acids. Expressing the results of elemental analysis in atomic percent gives the information about the changes that occur with humic substances during soil formation. Ratios H:C, O:C and C:N characterize the direction of transformation processes of humic acids under anthropogenic impact to the soil. The H:C ratio determines the degree of enrichment of the HA structure with aromatic fragments, O:C - the degree of oxidation, C:N reflects the role of nitrogen-containing components in the construction of humic acids (Orlov, 1990; Gasanova et al., 2018). In the arable soil of the long-term stationary experiment, where various methods of land use are studied, a low carbon content was found in HA structure of the soil under the crop rotation with legumes saturation– 42.8 percent (two fields of clover and lupine) - 28.35 at. percent (Table 2) and high hydrogen content –47.43 at. percent. In that variant, the maximum H/C ratio was noted, equal to 1.67, indicating the predominance of aliphatic groups in the structure of supramolecular HA aggregates. The same data were obtained by other researches (Popov, 2004; Milkheev, Tsybenov, 2018). The predominance of organic matter mineralization processes over the accumulation of humic substances was noted in the soil in grain crop rotation and under continuous barley, where the loss of carbon over 41 years of the experiment was 7-9 percent compared with the initial level when the experiment was founded. Sometimes the total loss may be about y 30% of initial soil organic carbon in the topsoil. (Seremešić et al, 2021). Under these conditions, during the destruction of organic material, aliphatic groups disintegrate rapidly and aromatic structural fragments with a higher carbon content remain. For these treatments, a lower H/C ratio was noted: 1.20–1.28 and that indicates the increase in the aromatic structures share in supramolecular associations when the soil is depleted by carbon and nutrients. A high degree of oxidation (W=0.23) of HA in grain crop rotation indicates the depth of humification of organic matter (Semenov et al., 2013; Gasanova et al., 2018).

Table 2. Elemental content of soil humic acids in long-term experiment with various types of land use

Variant	Content, %					Atomic ratios			Degree of oxidation, (*W*)
	C	H	O	N	S	H/C	O/C	C/N	
Continuous barley	48.56 33.36	5.17 42.65	41.36 21.33	4.18 2.47	0.76 0.20	1.28	0.64	13.5	-0.001
Grain crop rotation (0% of legumes)	46.60 33.47	4.67 40.29	44.60 24.05	3.36 2.07	0.48 0.13	1.20	0.72	16.2	0.23
Conventional crop rotation 28.6 % of legumes)	49.59 32.84	5.64 44.85	39.97 19.87	3.94 2.24	0.86 0.22	1.37	0.61	14.7	-0.16
Crop rotation (42.9 % of legumes)	43.93 28.35	6.12 47.43	45.15 21.87	3.79 2.10	1.03 0.25	1.67	0.77	13.5	0.13
Neglected field	40.34 29.50	4.57 40.13	50.86 27.92	3.67 2.30	0.58 0.16	1.36	0.95	12.8	0.53
Mixed forest	47.91 30,51	6.21 47.48	41.32 19.75	3.79 2.07	0.79 0.19	1,56	0.66	14.4	-0.26
Poaceous-forb meadow	49.11 32.07	5.83 45.73	40.02 19.62	4.22 2.36	0.84 0.21	1.43	0.61	13.6	-0.20

Footnotes:

* Above the line - mass fraction, below the line - atomic fraction (all calculations are given for ash free preparations)

The widest C/N ratio - 16.2, was found in the soil HA in the grain crop rotation, that showed their depletion with nitrogen, because they are formed from nitrogen-poor plant residues of grain crops (Orlov, 2019). The HA of neglected field soil were characterized by minimum content of hydrogen and maximum content of oxygen. The O/C ratio of humic acids in the considered variants increased with a decrease of the anthropogenic influence on the soil and was maximum in soil HA under neglected field – 0.95.

The oxidation of humic acids (W) varied from –0.26 to +0.53 owing to the type of land use. The maximum content of oxygen-containing groups was determined in HA of the neglected field soil. The high degree of HA oxidation, the positive sign of this indicator may serve as evidence of the most favorable conditions for the humification of organic matter in the soil under neglected field (Abakumov, 2009; Motuzova et al., 2012; Gorbov, Bezuglova, 2013).

Despite the fact that the total biomass of the forest biocenosis is much larger compared to the neglected field biomass, the soil under it can accumulate more carbon. This is owing to the alienation of biomass during timber harvesting. The cultivation of long cycle forest species, whose economic uses are not dedicated exclusively to wood production (fruit trees, rubber trees and so on) has comparative advantages in relation to the short-cycle tree species because carbon remains stored in the vegetation for longer periods (Torres et al., 2020).

The data of elemental analysis of HA from virgin sod-podzolic soil under forest and meadow indicate that under the conditions of leaching regime, low content of exchange bases, humic acids with a low content of carbon and nitrogen in supramolecular aggregates composition were formed from plant residues enriched with cellulose-lignin complex. Low microbiological activity led to decrease of organic matter (plant residues) mineralization, what led in turn to increase of aliphatic groups share, the H:C ratio was 1.43-1.56.

The conducted studies have showed that humic acids separated preparatively from the arable soil in long-term stationary experiment and its virgin analogues, correspond to the average values for the class of humic acids of sod-podzolic soils in terms of constitutional elements content (C, H, N, O). The aliphatic fragments dominated in humic acids structure, the C:H ratio was 1.20-1.67. The similarity of humic acids in arable and virgin soils resulted from almost the same set of formation factors: soil, plants, moisture, heat, microorganisms; the differences were in the qualitative composition of organic material in the soil and its quantity, different intensity of anthropogenic impact, and different random factors (Semenov et al., 2013).

Infrared spectroscopy is the obligatory and most important diagnostic method for studying humic substances. The method makes it possible to identify atomic groups and provides information about the type of chemical bonds and structural elements of humic acid molecules (Orlov,1990; Motuzova et al., 2012). The set and intensity of the absorption bands make it possible to estimate the role of aromatic and aliphatic fragments in the structure of supramolecular aggregates. It was found in the comparative study of the spectra, that humic acids from different types of soils have the same type of IR spectra, which allows to speak about the general way of their genesis. IR spectra are used as a characteristic diagnostic sign of humic acids, that makes it possible to reveal some features associated with the conditions of their formation (Shevtsova et al., 2019; Starykh et al., 2019; Zherebtsov et al., 2019).

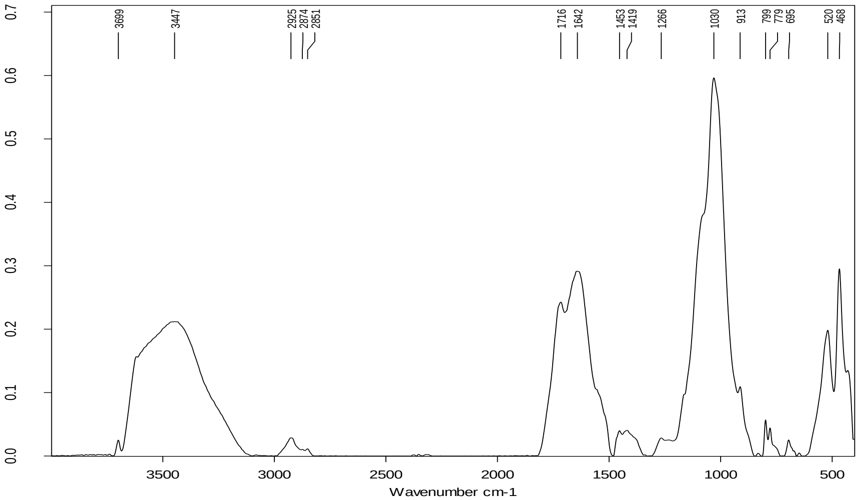

Figure 1. Infrared spectrum of humic acids in sod-podzolic soil under the mixed forest

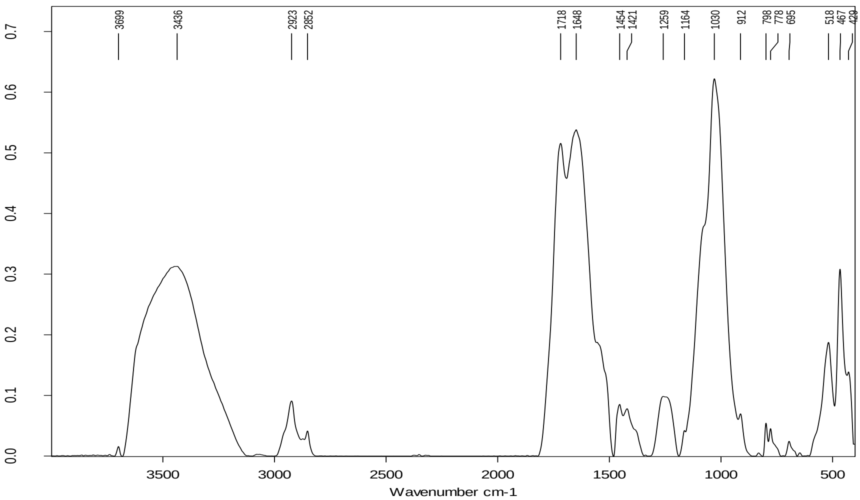

Figure 2. Infrared spectrum of humic acids in sod-podzolic soil under poaceous-forb meadow

The IR-spectra of humic acid preparations from the soil under mixed forest and poaceous-forb meadow are typical for sod-podzolic soils (Fig. 1, 2). They are characterized by a large set of absorption bands. Absorption bands in the region of 2800-3000 cm^{-1} were resulted from valence vibrations C-H of methyl (CH_3) and methylene (CH_2) groups. A stronger absorption in this region was observed in the spectrum of HAs in the soil under poaceous-forb meadow, what indicates a greater amount of aliphatic components of relatively low molecular weight in the structure of supramolecular aggregates compared with HA under the mixed forest. A wide absorption band in the zone of 3300-3500 cm^{-1} is responsible for hydrogen bonds. Intensive absorption in the region of 1700-1720 cm^{-1} was owing to wavering of the >C=O groups of carboxylic acids.

The presence of aromatic groups in HA aggregates was indicated by the absorption band at 1605-1670 cm^{-1}, which was owing to valence vibrations of conjugated double bonds of carbon atoms. The band (shoulder) in the region of 1510 cm^{-1} indicated the presence of aromatic C=C bonds in the macromolecule composition but its intensity was weak. Absorption in the zone of 1400–1470 cm^{-1} can be attributed to deformation vibrations of the C–H bond in CH_2 groups. Absorption bands with a maximum at 1200-1280 cm^{-1} were resulted from wavering of the C–O bond of simple ethers and similar compounds. It is possible that absorption in that region was caused by asymmetric valence vibrations in the C-O-C groups, which was confirmed by the characteristic symmetric vibrations of this group in the region of 1030 cm^{-1}.

The investigated humic acids of the soils in long-term experiment have absorption bands in a wide wavelength range from 500 to 4000 cm-1. The studied methods of land use had little effect on the presence of the most characteristic atomic groups and the intensity of the absorption bands resulted from the fluctuations of these groups (Fig. 3-7). The intensive absorption band at 3436-3465 cm-1 was owing to valence wavering of OH groups linked by intermolecular hydrogen bonds.

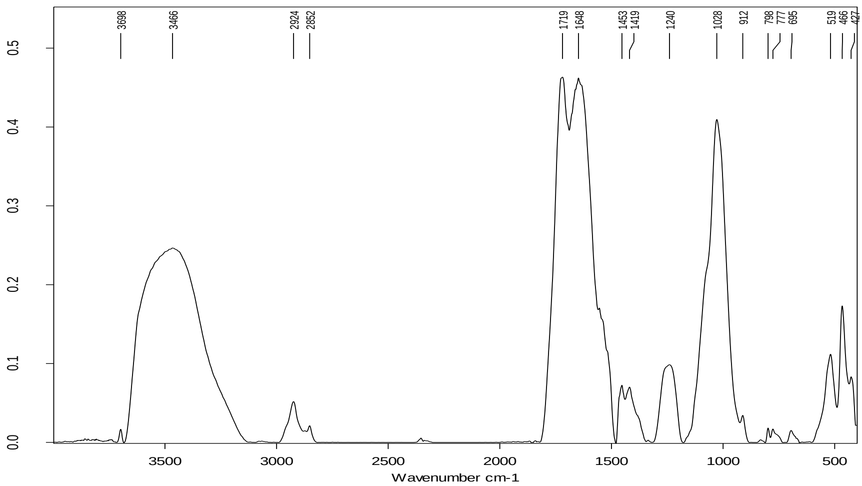

Figure 3. Infrared spectrum of humic acids in sod-podzolic soil under continuous barley

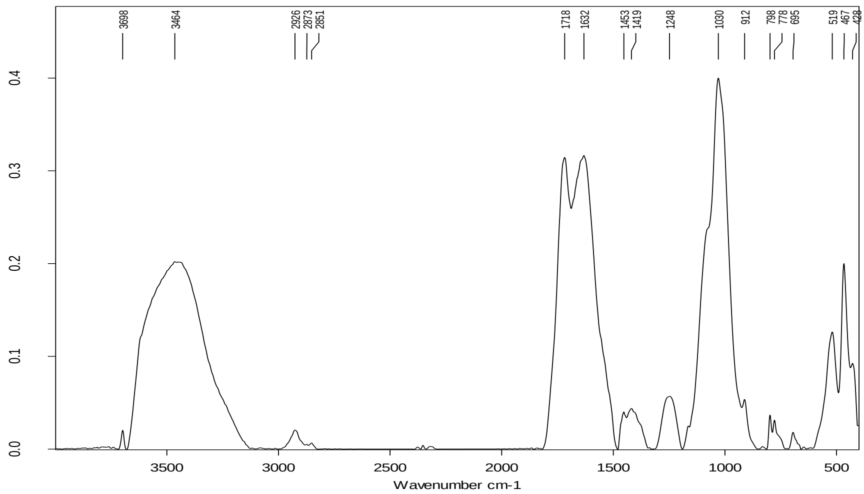

Figure 4. Infrared spectrum of humic acids in sod-podzolic soil under grain crop rotation (no legumes)

Absorption in that area was maximum for humic acids of the soil under typical crop rotation, minimum - for neglected field soil. Absorption bands (2929-2924 and 2849-2875 cm^{-1}) were resulted from C-H valence vibrations of methyl (CH_3) and methylene (CH_2) groups. These absorption bands proved themselves most intensively in the spectra of soil humic acids in typical crop rotation, which indicated the presence of sufficient amount of terminal methyl groups in the structure of these acids. The intensity of that band was about the same for the soil HA in the grain crop rotation and the crop rotation with high content of legumes, what can be explained by the destruction of aliphatic structures and, consequently, by decrease in the content of methyl and methylene groups in them. In the case of humic acids in typical crop rotation, the opposite trend was observed, that is, the absorption intensity of these bands increased due to content raise of aliphatic groups in them. The presence of the above groups was confirmed by absorption bands in the zone of 1454-1418 cm^{-1}.

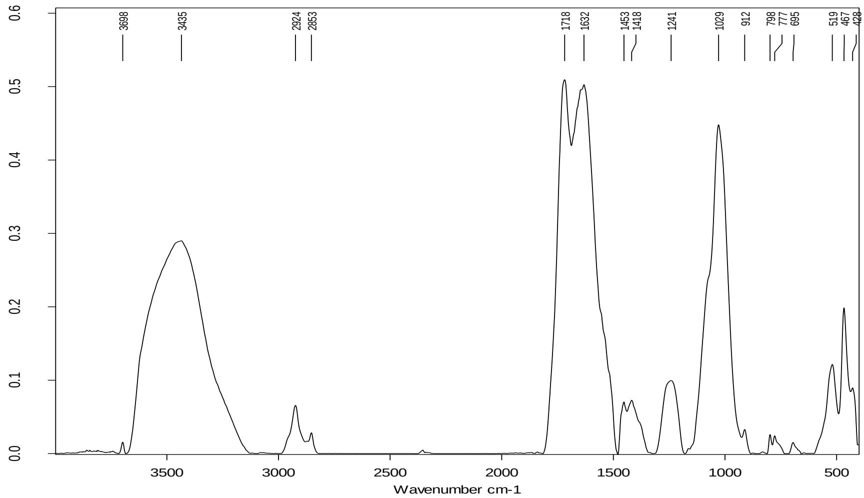

Figure 5. Infrared spectrum of humic acids in sod-podzolic soil under seven-course crop rotation

(28.6 percent legumes)

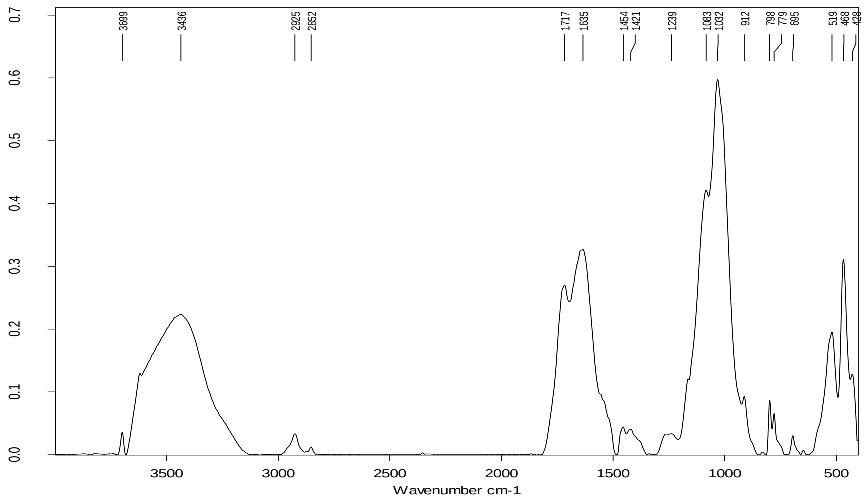

Figure 6. Infrared spectrum of humic acids in sod-podzolic soil under seven-course crop rotation (42.9 percent legumes)

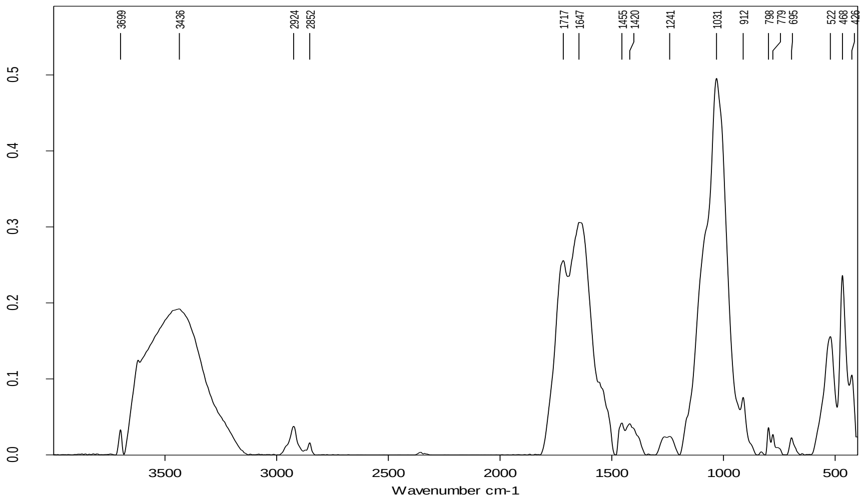

Figure 7. Infrared spectrum o f humic acids in sod-podzolic soil under neglected field

In the range of wave numbers 1800-1300 cm^{-1}, the most clear and intense were the absorption bands 1718-1719 and 1632-1647 cm^{-1}, which were present in humic acids in all variants. That may be explained by the presence of carboxylate ions and bending vibrations of NH_2 amides (Amide II band). The band at 1719–1717 cm^{-1} was owing to >C=O bond vibrations of carboxylic acids (Orlov, 1990).

The severity degree of this band affirmed that soil humic acids under the conventional crop rotation and continuous barley have the largest number of carboxyl groups in their content. The weakest fluctuations of the >C=O group of carboxylic acids were expressed in soil humic acids under crop rotation with a high (42.8 percent) share with legumes and in neglected field soil. The rest of the absorption bands can be associated with deformation vibrations of NH_2 amides (bands Amide I; Amide II) – compounds of amino acids type.

Based on the general form of the spectra, it can be assumed that the composition of humic acids of the studied variants contains nitrogen-containing structures of the amino acid type. The absorption band in the range of 1647-1632 cm^{-1} was owing to valence wavering of aromatic structures C=C group in supramolecular aggregates of humic acids. That band was most intensely expressed in the IR spectrum of conventional crop rotation, and the least in the spectrum of humic acids in neglected field soil. In the range of wave values 1300–500 cm^{-1}, the interpretation of absorption bands is rather difficult. In this area, oxygen-containing groups of various nature (alcohols, ethers, phenols), that is, OH groups, can appear. It can be assumed that within the wave numbers of 1000-1300 cm^{-1}, the absorption bands showed the presence of oxygen-containing groups of HA.

CONCLUSION

Different types of land using caused changes of different intensity and direction in condition of studied sod-podsolic soil. The humic acids separated from arable soil and its virgin analogs in long-term experiment, in terms of the content of basic elements (C, H, N, O, S), corresponded to the average values for humic acids in sod-podzolic soils. The carbon content in the HAs of arable and virgin sod-podzolic soil was 28.3-33.5, hydrogen 40.1-47.5, oxygen 19.6-27.9, nitrogen 2.1-2.5 and sulfur 0.1-0.2 at. percent. Aliphatic fragments predominated in the HA structure (H / C ratio - 1.20-1.67). Differences in the HA in arable and virgin soils were resulted from the qualitative content of organic matter input to the soil, its quantity and the intensity of anthropogenic impact. It was found that the saturation of field crop rotation with legumes up to 42.9 percent make it possible to increase the share of aliphatic groups in the structure of soil humic acids (the maximum H/C ratio of 1.67 was noted). The use of five-course grain crop rotation promoted to increase the ratio of stable aromatic structures (the minimum H/C ratio 1.20 was noted), that indicates the predominance of organic matter mineralization processes over the accumulation of humic substances. The depletion of the HA structure with nitrogen was noted in the soil under grain crop rotation, (the maximum C/N ratio was noted - 16.2). The O/C ratio of HA in the experiment increased with a decrease in the anthropogenic impact on the soil and was maximum in the soil HA under neglected field 0.95. There high degree of oxidation of humic acids (W=+0.53) was noted, what may indicate the most favorable conditions for the humification of organic matter. Humic acids separated from the soil in different treatments of long-term experiment had the same type of IR spectra. Based on the presence of absorption bands in the range 1718–1719 cm^{-1} (wavering of the C=O group of carboxylic acids) and 1632–1647 cm^{-1} (valence vibrations of conjugated double bonds), it can be assumed that aromatic structures were presented in supramolecular aggregates, what was more intensely expressed in the spectra of soil humic acids under conventional crop rotation and continuous barley.

The aggregates of soil humic acids in crop rotation soil with high saturation with legumes and soil under mixed forest had more branched structure, apparently, they consist from a larger number of individual monomers of various molecular weights with large number of aliphatic groups. That was confirmed by intense absorption in the range 2929-2849 and 1400-1470 cm^{-1}, caused by valence vibrations of methyl and methylene groups.

The predominance of organic matter mineralization processes over the accumulation of humic substances was noted in the soil under grain crop rotation and under continuous barley, where the loss of carbon over 41 years of the experiment was 7-9 percent compared with the initial level. The most favorable conditions for the humification of organic matter was noted in the soil under neglected field and mixed forest. The soil under conventional seven-course field rotation with legumes and under poaceous-forb meadow was characterized by relatively favorable parameters, these variants occupy an intermediate position among all studied treatments.

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GENOTYPE BY YIELD*TRAIT BIPLOT FOR GENOTYPE EVALUATION IN WINTER BARLEY

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ABSTRACT

Genotype selection in winter barley, considering multiple traits over several years, often faces challenges due to unpredictable rainfed conditions. The aim of this study was to utilize the genotype by yield*trait (GYT) biplot methodology for selecting genotypes using multi-trait data and analyzing trait profiles in winter barley under rainfed conditions in Southeast Bulgaria. In the experimental field of the Institute of Agriculture – Karnobat, Bulgaria 5 varieties and 15 advanced breeding lines of winter barley were evaluated in a complete block design with four replications in three growing seasons (2019/2020, 2020/2021, and 2021/2022). The GYT biplot method was used for ranking the genotypes based on their levels in combining grain yield with important grain quality traits. The variety Zemela (G5) demonstrated the most favorable combination of grain yield and grain quality. The advanced breeding line M102/10 (G20) outperformed not only the standard variety Veslets (G1), but also newly developed feed varieties like Bojin (G3), Bori (G4), and Zemela (G5), as indicated by the superiority index. These results highlight the potential usefulness of the GYT biplot for selecting genotypes with both desirable grain yield and crucial grain quality traits in winter barley, particularly under rainfed conditions.

Keywords: *winter barley, genotype by yield*trait biplot, multi-traits, superiority index, rainfed condition.*

INTRODUCTION

As barley is one of the most grown cereal in the world, it is crucial to develop high-yielding barley varieties with suitable grain quality to meet the specific end-use requirements. In Bulgaria, barley cultivation primarily relies on rainfed conditions, making the development of varieties with high and stable yield and tolerance to abiotic stresses one of the main challenges for barley breeding.

The simultaneous improvement of multiple traits of interest is one of the main hurdles in plant breeding. Improving a single trait is relatively straightforward however, it is unlikely to result in the development of a useful new variety. This task becomes particularly difficult when negative correlations exist between different traits, which may be attributed to gene linkage, pleiotropic effects, or population genetic structure.

The economic value of any trait in a barley genotype depends on the level of the main target trait, namely, grain yield (Yan et al., 2019). Therefore, the primary objective of multiple trait selection is to combine desirable traits with high grain yield within a single genotype. Various breeding approaches, such as independent culling, tandem selection, and index selection, have been employed to select genotypes with optimal combinations of grain yield and other agronomic traits, under both favorable and stress-inducing environmental conditions (Godshalk et al., 1988; Simmonds and Smartt, 1999; Bos and Caligari, 2007). However, these approaches often involve subjective decisions, as breeders assign weights or truncation points to each trait based on their judgment and the economic importance of the trait (Yan and Frégeau-Reid, 2018).

Recently, the genotype-by-yield trait (GYT) biplot approach, proposed by Yan and Fregeau-Reid (2018), has emerged as a valuable method for selecting superior genotypes based on multiple traits. The GYT biplot ranks genotypes according to their combination of yield with other target traits, such as grain quality and disease resistance. It provides multiple views that not only rank genotypes based on yield-trait combinations but also reveal the trait profiles of each genotype, highlighting their strengths and weaknesses. The GYT biplot approach has been effectively utilized for genotype selection across various cereal crops, including bread wheat (Hamid et al., 2019; Merrick et al., 2020), durum wheat (Kendal, 2019; Mohammadi, 2019; Faheem et al., 2022), and barley (Kendal, 2020; Karahan and Akgun, 2020; Hudzenko et al., 2021; Bakhshi & Shahmoradi, 2022).

The aim of this study was to utilize the genotype by yield*trait (GYT) biplot methodology for selecting genotypes using multi-trait and multi-year data and analyzing trait profiles in winter barley under rainfed conditions in Southeast Bulgaria.

MATERIAL AND METHODS

A total of twenty genotypes of 6-rowed feed winter barley were included in this study. The genotypes consisted of five varieties, namely Vaslets (G1), Izgrev (G2), Bojin (G3), Bori (G4), and Zemela (G5), and fifteen advanced mutant lines: M56/3 (G6), M56/9 (G7), M93/2 (G8), M93/5 (G9), M93/11 (G10), M93/14 (G11), M93/19 (G12), M100/2 (G13), M100/4 (G14), M100/7 (G15), M100/12 (G16), M102/1 (G17), M102/3 (G18), M102/5 (G19), and M102/10 (G20).

The study was conducted in three growing seasons 2019/2020, 2020/2021, and 2021/2022 at the experimental field of the Institute of Agriculture - Karnobat, Southeast Bulgaria (42°39' N, 26°59' E). The soil of the experimental field was slightly acid (pH is 6.2) Pellic Vertisol. The experiments were organized in a Complete Randomize Block Design with 4 replications on plots of 10 m^2 with sowing rate 450 seeds/m^2.

Protein content, % (Keldal's method); lysine content, % (ninhydrin method); fat content, % (Soxhlet method); ash content, % (after burning in a muffle furnace at

550 °C for 3 h); fiber content, % (Weende method), β-glucan content, % (Megazyme kit K-BGLU 07/11), 1000-grains weight, g were analyzed.
The data underwent graphical analysis using the Genotype × Yield × Trait (GYT) biplot method, following the approach outlined by Yan and Frégeau-Reid (2018). A superiority index (SI) combining all yield-trait interactions was calculated based on the standardized GYT data so that the mean for each trait or yield-trait combination was 0 and the variance a unit (Yan and Frégeau-Reid, 2018).
The GYT biplots were carried out in RStudio, R version 4.2.1 by using "metan" R package (Olivoto and Lúcio, 2020).

RESULTS AND DISCUSSION

Accordingly, Yan and Frégeau-Reid (2018) the GYT data was generated by the combination of each trait and grain yield (Table 1). This approach involves applying positive selection through multiplication or negative selection through division for a particular trait. Consequently, the selection outcomes in GYT biplot analysis strongly rely on the included traits. Therefore, Yan and Frégeau-Reid (2018) recommend incorporating only essential traits for the success of a variety in GYT biplot analysis. In present study 5 varieties and 15 advanced breeding lines of winter feed barley were tested. In addition to grain yield, which is of the utmost significance for feed barley, other essential grain quality traits were employed to assess the tested genotypes. Therefore, the GYT table was obtained as follows: for the protein, starch, lysine, fat, ash content, and 1000-grains weight the values for each trait was multiplied (*) with the yield. While for traits fiber and β-glucan content, in which the high values are undesirable, the value for each trait was divided (/) by the yield. The GYT biplot graphically displays the standardized GYT data (Table 1), and the different views of the GYT biplot (Figs 1, 2, and 3) allow the data to be investigated from different angles. The GYT biplot represented 82.23% of total variation by plotting first two principal components.
The Superiority Index (SI) enables the assessment and ranking of genotypes based multiple traits. In this study, the superiority index identified Zemela as exhibiting the most favorable combination of grain yield and quality among the tested varieties. Furthermore, when comparing the advanced breeding lines to the standard variety Veslets (G1), the line M102/10 (G20) overperformed not only the standard variety but also the newly developed feed varieties such as Bojin (G3), Bori (G4), and Zemela (G5), as indicated by the superiority index.

Table 1. Standardized genotype by yield*trait (GYT) data and superiority index for the genotypes

Genotypes	GY*P	GY*L	GY*A	GY*F	GY/Fb	GY/G	GY* TGW	SI
G1	0.54	0.17	-0.36	1.22	0.15	0.13	-0.48	1.37
G2	-1.30	-0.82	-1.40	-0.91	-0.73	-1.17	-1.39	-7.73
G3	1.01	1.45	1.18	0.02	2.08	1.46	0.72	7.92
G4	0.88	0.88	0.63	-0.09	-1.00	1.28	-0.08	2.51
G5	1.15	1.50	0.93	1.40	1.60	1.10	0.60	8.28
G6	0.01	0.26	0.98	0.54	1.41	-0.20	0.32	3.32
G7	0.70	-0.06	0.25	0.24	-0.24	0.25	0.47	1.61
G8	0.32	0.21	0.39	-0.13	0.00	0.54	-0.13	1.19
G9	-0.78	-1.09	-0.56	-1.55	-1.51	-0.89	-1.08	-7.46
G10	-0.72	-0.11	-0.89	0.00	-0.32	-0.70	0.11	-2.63
G11	-1.35	-1.35	-1.46	-0.93	-0.78	-1.30	-0.69	-7.86
G12	-2.38	-2.29	-2.25	-2.48	-1.55	-2.19	-2.48	-15.63
G13	-0.27	-0.14	-0.37	0.19	-0.51	-0.46	-0.47	-2.03
G14	0.25	-0.13	0.08	0.18	0.07	0.00	0.23	0.68
G15	0.36	0.38	0.50	0.43	0.23	0.56	0.86	3.32
G16	-0.14	-0.63	-0.06	-0.22	-0.16	0.10	-0.41	-1.52
G17	0.37	0.98	1.16	1.22	1.01	0.03	1.25	6.04
G18	-0.54	-0.65	-0.36	-0.07	-0.62	-0.47	0.19	-2.53
G19	-0.11	-0.23	-0.07	-0.69	-0.26	0.01	0.22	-1.13
G20	1.99	1.65	1.69	1.64	1.14	1.93	2.22	12.27

P - protein content, %; L - lysine content, %; F - fat content, %; A - ash content, %; Fb - fiber content, %; G - β-glucan content, %; TGW - 1000-grains weight, g; GY – grain yield, t/ha; SI - Superiority Index

Due to the fact that all yield-trait combinations in the GYT biplot included grain yield as a component, most of them were positively associated as exhibited by the acute angles in the biplot (Figure 1). Nevertheless, some of the strong associations between traits, such as the negative correlation between fiber and β-glucan content, can be observed in the GYT biplot, as indicated by the magnitudes of angles between GY/G and GY/Fb. (Figure 2).

Figure 3 presents the polygon view, also known as the "which-won-where" analysis, illustrating the relationship between different yield-traits combinations and genotypes. This visualization technique allows us to identify genotypes that exhibit superior performance in combining grain yield with other desirable traits.

Upon analyzing the figure, it is evident that genotype G17 stands out as it possesses the largest value for GY/G, indicating an exceptional combination of

high grain yield and low β-glucan content. This suggests that G17 is a promising variety for achieving both high yield and reduced levels of β-glucan, which is desirable for feed barley.
Furthermore, genotype G4 demonstrates a favorable combination of high grain yield and low fiber content in the grain. This characteristic is valuable as low fiber content is often associated with improved feed grain quality.
Another noteworthy genotype is G20, which exhibits high levels across most of the studied yield-traits combinations. This implies that G20 possesses a well-rounded profile, excelling in both high yield and grain quality simultaneously.
Ranking of the genotype by yield*trait biplot in Figure 3 ranks the genotypes based on their overall superiority. The ATA axis separates genotypes that perform better than the average (placed on its right) from those that perform poorer than the average (placed on the left side) (Yan & Frégeau-Reid, 2018). Based on this ranking, the best-performing genotypes in terms of yield-trait combinations are G20, G5, G7, and G3, while genotypes G12, G2, G9, and G11, positioned on the far-left side of the biplot, are ranked as the worst performers.
Genotypes placed close to the ATA axis tend to exhibit balanced trait profiles, suggesting they have relatively equal strengths and weaknesses across the studied traits (Yan & Frégeau-Reid, 2018). In this case, G20, G5, and G7 demonstrate balanced trait profiles.
Furthermore, genotypes positioned above the ATA axis, such as G7, G3, G6, G10, and G11, showed a relatively better combination of 1000-grain weight (TGW) and lower β-glucan content (G). Conversely, genotypes placed below the ATA axis, including G4, G1, G15, G16, and G9, exhibit a better combination of grain yield, higher protein content (P), and lower fiber content (Fb).
The results showed that the GYT biplot approach allows the selection of high-yielding barley genotypes with good agronomic characteristics and it may be used to assist the development of new barley varieties with high economic value for the rainfed conditions of Bulgaria.

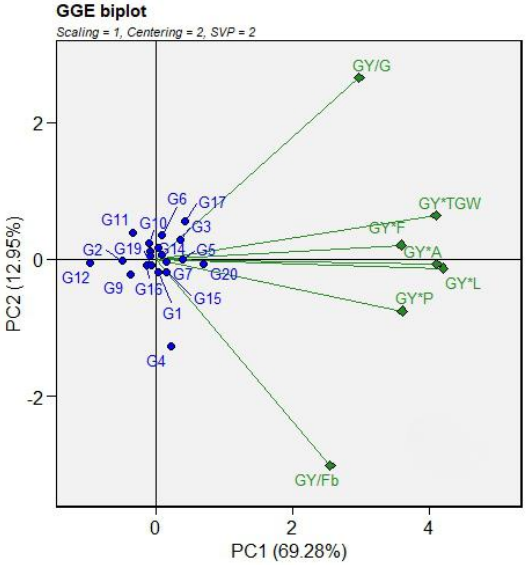

Figure 1. The Tester Vector view of the genotype by yield*trait (GYT) biplot

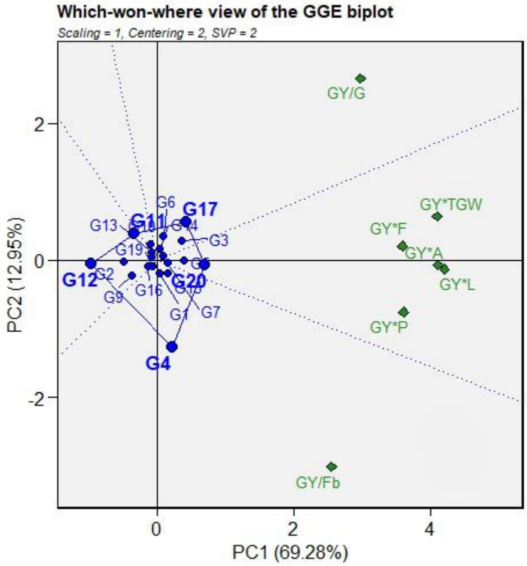

Figure 2. The which-won-where view of the genotype by yield*trait (GYT) biplot

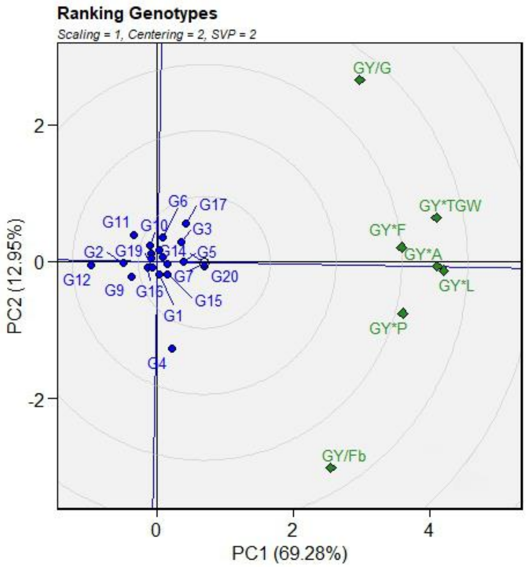

Figure 3. Ranking of the genotypes by yield*trait (GYT)

CONCLUSIONS

The results of this study provide valuable insights into the assessment and selection of winter feed barley genotypes based on grain yield and feed grain quality traits using the GYT biplot analysis. Among the tested genotypes, Zemela (G5) exhibited the most favorable combination of grain yield and quality. The advanced breeding line M102/10 (G20) displayed superior performance not only compared to the standard variety Veslets (G1) but also outperformed newly developed feed varieties, including Bojin (G3), Bori (G4), and Zemela (G5), as indicated by the superiority index. The results highlight the potential of the GYT biplot approach in selecting high-yielding barley genotypes with desirable agronomic characteristics. This methodology can support the development of new barley varieties that possess high economic value, particularly suitable for rainfed conditions in Bulgaria.

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TREATED WASTEWATER IRRIGATION: EXPERIMENTAL RESULTS AND LESSONS LEARNED

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ABSTRACT

The identification and introduction of new, alternative sources of water for irrigation is a growing trend in many countries around the world, including Slovenia. To obtain larger quantities of clean drinking water, the use of treated wastewater (TWW) for irrigation can reduce the consumption of the primary natural water resource. Irrigation with TWW is increasing due to population growth and the associated increase in wastewater volumes, as well as the need to adapt to climate change. In order to study the impact of using wastewater for irrigation on soils, plants and crops, an experimental field with 30 lysimeters was established in the Central Municipal Wastewater Treatment Plant (CMWWTP) in Ajdovščina, Slovenia. In this paper, we present the results of the first experiments with pumpkin conducted at the Ajdovščina WWTP. We investigated the effects of irrigation with differently treated irrigation water sources from the WWTP compared to irrigation with tap water as a control. Presented results are outcomes of a short-term experiment. To fully understand and investigate the effects of TWW application on soils, plants and yields the study should include multi-year trials and more frequent irrigation.

Keywords: *irrigation, water source, treated wastewater, pumpkin, soil.*

INTRODUCTION

Water is an extremely important natural resource. Agriculture is one of the main consumers of water, accounting for 80% of total water use according to current estimates, with water use for irrigation expected to increase by an additional 15% by 2030 (SDG Report, 2019). In order to obtain more clean drinking water, new alternative sources of water for irrigation are currently actively sought and introduced around the world. One of these is the use of TWW. TWW is defined as "water that has undergone at least secondary treatment and disinfection and is reused after leaving the treatment plant" (Hashem and Qi, 2021). It can be used for irrigation of agricultural and other areas (environmental and recreational), for industrial reuse, or for municipal purposes (urban sanitation and firefighting). De

Carlo et al. (2020) state that the use of TWW as a source for irrigation has economic and environmental benefits. The need to apply certain types of fertilisers to the soil is reduced or even eliminated because it contains organic matter and nutrients that are beneficial to plants. However, TWW use also poses risks, as it can increase soil salinity and increase the levels of microorganisms and other modern pollutants in the soil (Gao et al., 2021; De Carlo et al., 2020; Ganjegunte et al., 2018).

Water resources for irrigation are limited in many places, both in Slovenia and around the world. Therefore, in areas where there are no other water sources, the use of TWW for irrigation could be a new, alternative water source. However, irrigation with TWW requires a number of additional restrictions and quality controls of the TWW used, which are not required when using conventional water sources for irrigation. To test the suitability and find new water sources for irrigation, we are conducting experiments presented in this paper and planning new ones for the future.

In our study, we aimed to determine the suitability of TWW (application and comparison of different TWW treatment methods) for irrigation at the Ajdovščina (Slovenia) waste water treatment plant (WWTP) lysimeter station. Authors were also interested in the concentration of heavy metals in soils and plants irrigated with TWW, and the influence of TWW on the electrical conductivity (EC) and pH of the outflow from the lysimeters.

MATERIALS AND METHODS

The Ajdovščina WWTP in Slovenia operates as a conventional flow-through system with pre-denitrification and anaerobic stabilisation of the sludge in the digesters. It consists of two lines, a water line and a sludge line. In addition to the installations that is part of the conventional WWTP an algae system and a constructed wetland are also installed at the location.

The study on irrigation with different TWW was conducted as a block experiment with five treatments in six replicates. The treatments consisted of four different irrigation water sources: water from a constructed wetland (CW), water treated with algae technology (AT), water from the WWTP with additional fertilisation (WWTPf) and water from the WWTP without fertilisation (WWTPnf). As the fifth treatment, tap water as control (C) was used. The experiment was carried out in 30 lysimeters buried in the soil with a size of 400 mm × 1000 mm, each equipped with two soil sensors for water content and salinity of the soil. The lysimeters were filled with previously analysed soil from the area surrounding the WWTP. The pH and EC of the different water sources used for irrigation were also analysed. A plant of Hokkaido pumpkin *(Cucurbita maxima Duchesne)*, cv. 'Shishikura', one of the most important pumpkin species for agriculture, was planted in each lysimeter. Hokkaido pumpkins were planted on May 31, 2019, and fertilised with 31 g KAN (39%), 44 g of Hypekorn fertiliser (0:26:0), and 84 g K2SO4 per plant. Additional fertilisation was applied on June 21, 2019 and July 24, 2020 with 31 g KAN per plant.

Irrigation was carried out according to the needs of the plants using available data for reference evapotranspiration. Which was 487.4 mm during the growing season, and precipitation, where 169.4 mm of precipitation fell during the experiment according to the nearest weather stations. Irrigation was carried out at an interval of three days. It was less than the above calculation required, but there were no visible signs of water deficit on the plants. The first week, irrigation was manual (13.6 l/plant), then drip irrigation (one drip per plant/lysimeter) at seventeen rates (68 l/plant) was used. The pH, EC, and volume of water draining from the lysimeters were measured in the lysimeter outflow between July 10, 2019 and August 28, 2019. Harvesting of the crop (pumpkins), plants, and soil samples was conducted on September 3, 2019.

RESULTS AND DISCUSSION

Concentrations of metals arsenic (As), cadmium (Cd), copper (Cu), molybdenum (Mo), nickel (Ni), lead (Pb), zinc (Zn), cobalt (Co), and chromium (Cr) in soil were monitored. The metal content in soils is mainly influenced by the soil parent material, atmospheric deposition, manure and slurry application, various plant protection products and mineral fertilisers.

Most of the metals analysed, with the exception of Cu and Ni, did not exceed the immission limit values (Official Gazette of the Republic of Slovenia No. 68/96 dashed line, Figure 1), and there were no statistically significant differences in the average metal content in soil between treatments (Figure 1). Albdaiwi et al. (2022) and Kumar et al. (2021) confirmed similar results and concluded that continuous irrigation with TWW did not lead to accumulation of metals in soil and did not exceed the World Health Organisation (WHO) limit values. Higher Cu concentrations in the soil may be due to human influences (plant protection measures in the cultivation of vines and peaches in the area of the Ajdovščina WWTP) (Murtaza et al., 2015; Zupan et al., 2008). Higher Ni values are attributed to naturally high soil concentrations resulting from rock weathering in the area where the experiment was conducted (Zupan et al., 2008).

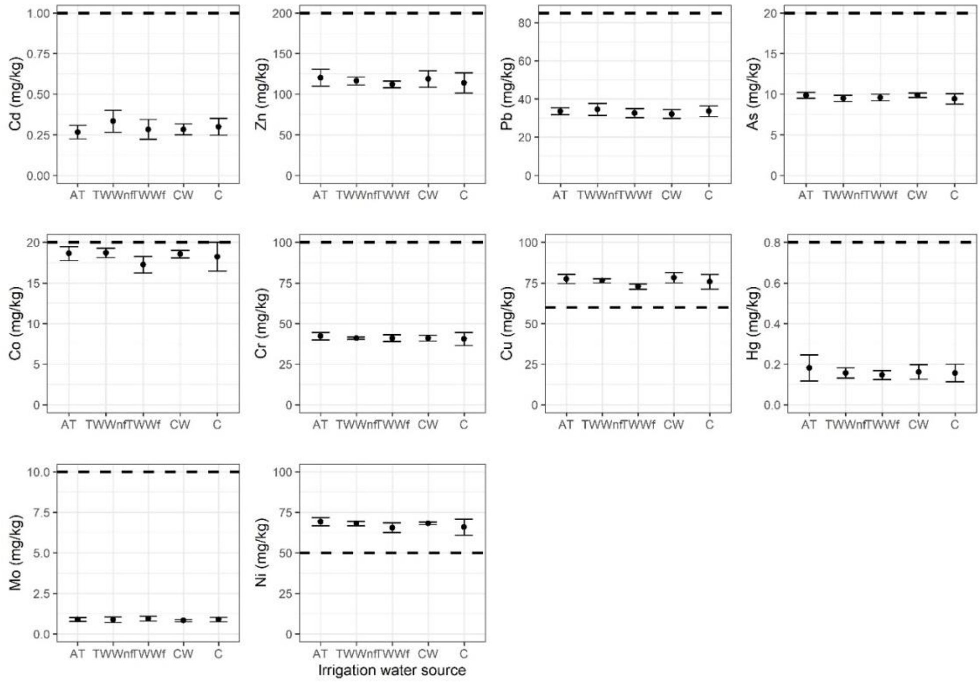

Figure 1: Average metal contents in lysimeters soils (mg/kg) for different irrigation water used with associated 95% confidence intervals at the Ajdovščina WWTP, sampled on 3 September 2019. The dashed line represents the immission limit value for metals in soil (Official Gazette of the Republic of Slovenia No. 68/96).

Metal content was also measured in samples from different parts of the plant. These data were analysed as a two-factorial experiment, with irrigation water representing one of the factors studied and the plant part representing the other. The results of the statistical analysis showed that there was no interaction between the irrigation method and the accumulation of metals in different parts of the plant (Figure 2). For all metals, there were statistically significant differences in the average metal content between the different plant parts. This was confirmed also in other studies, where Ofori et al. (2021) and Roba et al. (2016) found that plants and different plant parts have different bioaccumulation capacities. This mainly depends on the type of plant, the type of metal, and the type of soil. The accumulation of metals in plants and their transfer through the food chain to the human body represents the main risk for the development of various diseases when contaminated plants are consumed (Rai et al., 2019). Ofori et al. (2021) state that irrigation with TWW can increase the content of some metals in soils and plants, and their accumulation can lead to toxicity when concentrations are above limits.
Current dietary guidelines prescribe limits for metal concentrations in pumpkin fruit only for Cd and Pb. Mean Cd and Pb levels were statistically significantly lowest in fruit, but the confidence interval for these mean values included the 0.05 mg/kg fresh weight limit for fruit according to current Food and Agriculture

Organisation (FAO) and WHO (2019) dietary guidelines. Mean Zn and As content was statistically significantly higher in roots than in fruits and green parts, which were not statistically significantly different. Mean Co content in pumpkin fruit was statistically significantly lower than in the other two plant parts. The fruits contained more Cr, Cu, Mo and Ni on average than the roots and green part. Regarding the used irrigation water sources used for irrigation, statistically significant differences were found only for Cu and Mo. For Cu, higher contents were found on average when irrigated with TWWnf compared with TWWf and C. For Mo, on the other hand, the average content was higher with AT than with TWWf. The literature also suggests that metal contents can vary significantly in different plant parts irrigated with TWW. Hussain et al. (2019) and Kumar et al. (2021) indicate that several factors influence the transport and accumulation of metals in different plant parts. These include the concentration of these metals in the soil and the soil type, as well as the time of harvest and the stage of fruit maturity.

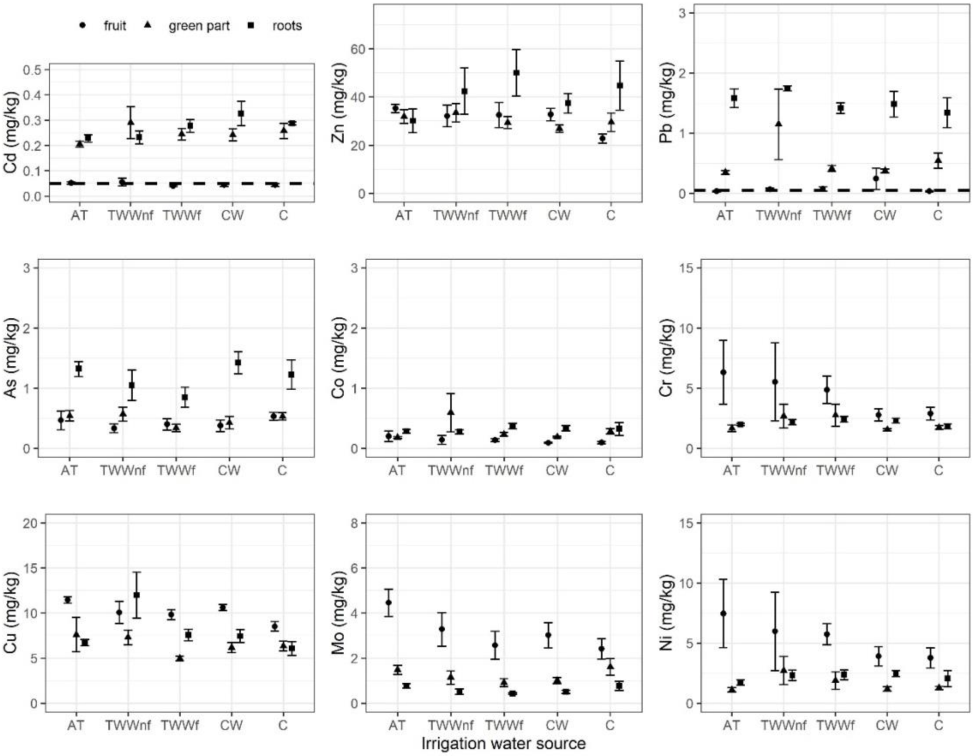

Figure 2: Mean metal contents (mg/kg) with associated standard error in roots, fruits, and the green part of the plant (stems and leaves) for different irrigation water source used. The dashed line represents the currently applicable limits for metal content in pumpkin fruits for human consumption (FAO/WHO, 2019).

In addition to monitoring metal accumulation in soil and in different parts of the irrigated crops, it is also necessary to study the EC and pH levels in the TWW. EC is an important indicator of irrigation water quality and a limiting factor for the choice of crops to irrigate, since some crops are more or less sensitive to EC (Drechsel et al., 2022). Therefore, we monitored EC and the pH of the outflow from the lysimeters and determined if there were differences between the different TWW sources. Figure 3 shows that the pH of outflows from the lysimeters does not differ by irrigation water source, although the irrigation waters used have different pH. The most likely reasons for this are the precipitations during the experiment, which had a dilution effect, and the fact that the outflow must pass through the entire length of the lysimeter.

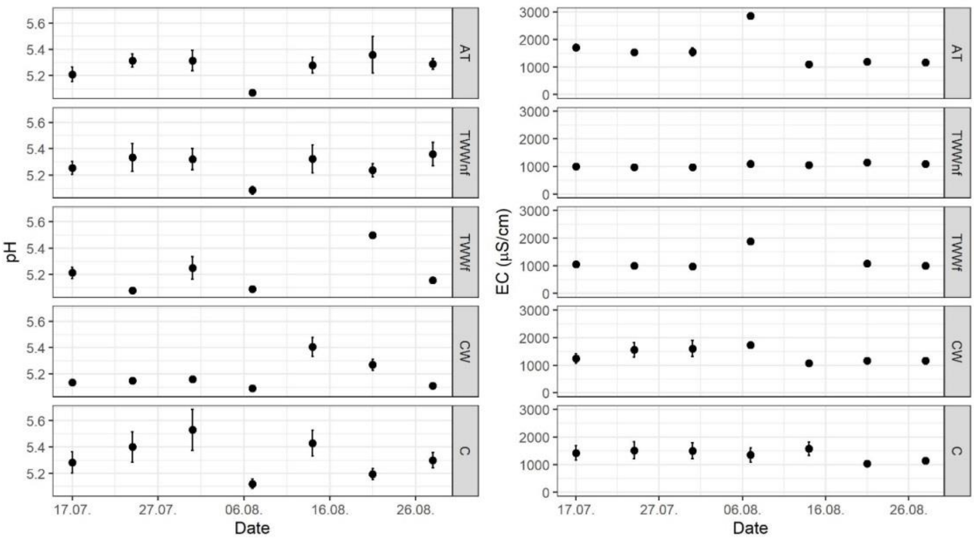

Figure 3: Time series of measurements of mean pH (left) and EC (µS/cm) (right) with associated standard error at the outflow of the lysimeters irrigated with different TWW sources, from July 17 to August 28, 2019.

CONCLUSION

After three months of irrigation, we found that there are no differences between irrigation with different TWW sources in metal concentrations and different plant parts, and there are no statistically significant differences between treatments in pH and EC values, while the treatment with TWWnf is statistically significantly lower than the treatments with C and AT. Our results are outcomes of a short-term experiment. To fully understand and investigate the effects of TWW application on plants, yields, and soils, the study should include multi-year trials and more frequent irrigation. Results show that the concept and practice of using TWW for irrigation has both advantages and disadvantages. There are advantages to using TWW for irrigation, but there are also environmental, health, and economic challenges. From our experimental experience, the main disadvantages are the risk of soil salinization and the increase of metal concentrations in the soil. However, the protection and saving of water resources, the supply of nutrients and the impact on farm profitability are the main advantages of irrigation with TWW.

Irrigation with TWW has impacts on soils, water resources, and public health. However, the nature and severity of the impacts depend not only on the quality of the TWW, but also on the characteristics of the irrigated soils, the morphology and physiology of the irrigated crops, the irrigation method and farming practices and the climate. Nevertheless, the use of TWW for irrigation can contribute to sustainable water use and lead towards the promotion and development of sustainable agriculture.

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CUSTODIAN FARMERS OF BAMBARA GROUNDNUT AND SORREL SEEDS IN MOSSI AREA OF BURKINA FASO: PROFILE, DIVERSITY AND CONSERVATION METHODS

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ABSTRACT

The conservation and preservation of landraces genetic resources are of utmost importance in securing sustainable food security, especially in the face of significant global changes. This study aimed to identify and profile custodian farmers dealing with the preservation of two neglected and underutilized crops, Bambara groundnut/ (*Vigna subterranean*) and sorrel (*Hibiscus sabdariffa*), and explore their conservation strategies in Burkina Faso. Through gender-disaggregated focus group discussions, 68 custodian farmers were identified across 11 villages, with a majority being female. Individual semi-structured interviews were then conducted to gain insights into the seed conservation techniques employed by these farmers. The study revealed that custodian farmers employed various techniques, such as utilizing cooking pot, canaries or ash, to conserve seeds. Seed exchange emerged as a common practice among custodian farmers, playing a significant role in enriching seed diversity and fostering social cohesion within local communities. Among the identified custodian farmers, 40 were conserving Bambara groundnut seeds, while 28 were custodians of sorrel seeds. They range in age from 30 to 105, and some of them have owned their landrace for over 80 years. Nineteen (19) landraces of Bambara groundnut and six (06) landraces of sorrel were identified across the villages. Overall, these findings underscore the necessity for further research to better comprehend the factors influencing the distribution and maintenance of traditional landraces as well as the diversity of conservation methods used. They also emphasize the importance of developing effective conservation strategies that engage local communities to ensure global food security.

Keywords: *biodiversity conservation, custodian farmers, crop diversity, orphan crops, Burkina Faso*.

INTRODUCTION

Conserving the genetic diversity of crops is crucial to ensuring food security for rural populations and promoting agricultural sustainability. The quest for increased agricultural productivity through intensified farming practices has led to a preference for monoculture and the genetic enhancement of a limited number of species and varieties (Maetz, 2013; Bhandari et *al*., 2017; Baa-Poku et *al*., 2020). Consequently, there has been a noticeable decrease in crop diversity, with the potential negative impacts further exacerbated by climate change (FAO, 2010). Climate change threatens the conservation of agricultural species as a whole, but it poses an even greater risk to neglected and underutilized species (NUS). These NUS, however, hold significant importance in the diet and socio-cultural life of rural communities and have proven to be more resilient in their local environments compared to exotic species (Lagacé et *al*., 2015).

Among these NUS, Bambara groundnut (*Vigna subterranea*) and sorrel (*Hibiscus sabdarifa*) are particularly noteworthy due to their significant agronomic, economic, and nutritional importance in Burkina Faso.

Vigna subterranea (L.) Verdc. is a leguminous crop species belonging to the *Papilionaceae* family (Baudoin and Mergeai, 2001). It is believed to have originated from Africa, possesses drought tolerance and requires minimal inputs for cultivation. Generally, Bambara groundnut cultivation is carried out as monoculture, however, it can also be grown in association with other crops, especially cereals such as millet, maize, sorghum, fonio, cotton, cowpea, okra, peanuts, cassava, sesame and hibiscus (Ouédraogo et al., 2013; Ouoba et al., 2016). The crop is not commonly affected by diseases and pests within its growing area, although it can be susceptible to different diseases (such as fungal and viral ones) and attacked by insects and nematodes under certain conditions (Nadembèga, 2016).

Hibiscus sabdariffa L. belongs to the Malvaceae family. The species of the Hibiscus genus are distributed in six sections, with *Hibiscus sabdariffa* belonging to the Fucaria section. According to Murdock (1959), West Africa is the centre of origin of Hibiscus sabdariffa. Many authors (Wilson and Menzel, 1964; Boulanger et al., 1984; McClintock and El Tahir, 2004) support Murdock's hypothesis by stating that *Hibiscus* in the Fucaria section, including *Hibiscus cannabinus* and *Hibiscus sabdariffa*, are native to sub-Saharan Africa. Commonly known as Guinea sorrel, it is mainly grown during the rainy season for its leaves, calyces, and seeds. Its cultivation in the dry season in market gardens is mainly aimed at providing leaves used as vegetable condiments (Ouangraoua et al., 2021). Guinea sorrel cultivation faces several problems, including diseases and pests. *Hibiscus sabdariffa* is susceptible to most diseases affecting cotton and okra, which are all in the same family, Malvaceae (McClintock and El Tahir, 2004).

The long-term conservation of seeds of these two neglected crops remains a significant challenge for farmers in Burkina Faso. Some of these farmers stand out for their commitment and ability to conserve a large diversity of traditional seeds of these NUS, including rare and/or endangered species/varieties. They are also the custodians of the intergenerational endogenous knowledge associated with the cultivation, conservation and use of these neglected species. The characterization of these custodian farmers and the conservation methods they use, and identifying the associated knowledge, could help to develop innovative conservation methods in order to improve farmers' resilience to climate change.

Through a combination of focus group discussions and semi-structured individual interviews conducted in 11 villages in Burkina Faso, this study aims (i) to identify farmers who are recognized by the community for their distinct commitment to cultivating a wide range of diverse and rare crop landraces of Bambara groundnut and Sorrel, (ii) to characterize these unique farmers and gain insights into their profiles, (iii) to document the landraces of Bambara groundnut and sorrel held by these custodian farmers, and (iv) to document the methods employed by them for conserving traditional Bambara groundnut and sorrel seeds.

MATERIAL AND METHODS

Study area

The study was conducted in four provinces in central Burkina Faso, including 11 villages (Table 1). The area is characterized by low rainfall and a short rainy season. According to the geographical distribution of ethnic groups, the study area is located in the homogeneous zone occupied by the Mossi ethnic group. Some villages (Koubri, Loumbila and Pabré) are close to Ouagadougou, the capital city, and are influenced by urbanization, while others are far from the capital and are typical of rural areas.

Table 1. Study sites.

Provinces	Departments	Villages
Kadiogo	Loumbila	Loumbila
	Koubri	Koubri
	Pabré	Pabré
Oubritenga	Ziniaré	Songpelcé
		Kolguiguessé
Bazèga	Kombissiri	Goundrin
	Saponé	Godin
Boulkiemdé	Pella	Pella
		Pelbilin
	Kokologo	Nidaga
		Meninga

Data collection

Two separate focus group discussions (FGDs) were organized in each village, one for men and one for women. The number of participants was 30-50 per focus group. During these FGDs, the concept of custodian farmers was explained to the participants as farmers who distinguish themselves from others through their commitment to conserving a wide diversity of crops, including rare landraces. Participants were then asked to suggest names of individuals whom they considered as custodian farmers. These discussions led to debates, resulting in the unanimous nomination of individuals by the FGD participants as custodian farmers.

The individuals proposed by the community were subsequently individually interviewed using a semi-structured interview approach conducted by pairs of interviewers. A pre-established individual survey questionnaire was employed to gather qualitative and quantitative data on various aspects, including: gender, age, religion, profession, education, whether the custodian farmer is a traditional practitioner or not, species maintained by each custodian farmer, seed conservation methods employed, duration of ownership of the conserved landraces, reasons for recognition by the community as custodian farmer, origin (allochthonous or indigenous) of the custodian farmer, seed conservation techniques used, mode of seed conservation, duration of conservation, cultivation of improved landraces or not and for what reason, seed replacement in case of loss, seed exchange. Prior to the formation of focus groups and individual surveys, consent was obtained from the village authorities through their signatures.

Data Analysis

Data analysis was conducted using R (version 4.1.0) statistical software, with descriptive statistics calculated for all variables including means, medians, standard deviations, frequencies, and percentages. Correlation analyses were carried out to explore associations between variables. Chi-square tests and analysis of variance were employed to investigate the relationships between different variables, with the significance level set at $p < 0.05$. Non-parametric Kruskal-Wallis ANOVA was used to determine the significance of differences, and multiple comparisons were conducted using the pairwise Wilcoxon test and adjusted according to the Holm method.

RESULTS AND DISCUSSION

Gender dynamics and education levels among custodian farmers

The study findings underscored the significant contribution of women as seed custodian farmers for minor crops in the studied area. Women played a crucial role in preserving the genetic diversity of sorrel and Bambara groundnut crops, demonstrating their active participation in sustainable agricultural practices and biodiversity conservation. The results revealed notable gender disparities among seed custodian farmers, with females constituting the majority at 75.4%, while males represented 24.6% of the total.

Education levels among seed custodian farmers were relatively low, with 94.2% identified as illiterate. This suggests potential challenges in accessing and applying modern agricultural knowledge and practices. However, a small proportion (5.8%) reported having attended school, indicating the potential influence of formal education on seed custodian practices.

For female custodian farmers, the mean age for those preserving the sorrel was 48.462 years, with a standard deviation of 7.875. The age range was from 30 to 68 years, with the majority falling within the interquartile range (Q1 = 43.0, median = 47.5, Q3 = 51.75). Among male custodian farmers, there were only two individuals preserving the sorrel species, with a mean age of 49 years and a small standard deviation of 1.414. The ages of these male guardians ranged from 48 to 50 years.

Although there is a global association between age and gender (chi-square test, p = 0.002), there is no significant difference observed within each species.

Bambara groundnut traditional Landraces

The custodian farmers use morphological characteristics such as colour, size, shape, or seed origin for the naming of traditional landraces. Based on the farmers' identification criteria (seed coat colour, seed size, plant lifecycle, and seed origin), a total of 19 landraces of Bambara groundnut were described by the custodian farmers across all villages. The most commonly encountered landraces are those whose description is based on seed coat colour. Custodian farmers give names to each landrace, but the same landrace can have different names in different villages. Conversely, different traditional landraces can have the same name in different villages. For example, the white-seeded Bambara groundnut (two seeds per pod) is called soum-kamana in the village of Songpelsé and raboega in the village of Pabré.

The mean age for female custodian farmers was 51.462 years, with a higher standard deviation of 14.564. The age distribution spanned from 30 to 103 years, with the central tendency shown by the interquartile range (Q1 = 42.25, median = 49.5, Q3 = 56.75). Among male seed custodians preserving the Bambara groundnut species, there were 15 individuals. Their mean age was 59.800 years, with a relatively larger standard deviation of 18.300. The ages ranged from 37 to 105 years, reflecting a wider spread of age among male custodians. Female seed custodians had a mean conservation duration of 30.154 years, with a slightly higher standard deviation of 17.417. The range spanned from 8 to 72 years, and the interquartile range showed that 50% of female custodians conserved the landrace for a duration between 24 and 33 years. Among male seed custodians preserving the Bambara groundnut species, the mean conservation duration was 19.867 years, with a larger standard deviation of 27.100. The conservation periods ranged from 7 to 114 months, and the quartile values indicated that 50% of male custodians conserved the landrace for a duration between 9 and 12 years. Concerning the duration of seed ownership, female seed custodians had an average ownership duration of 18.346 years, with a higher standard deviation of 17.283. The ownership periods varied from 3 to 80 years, and the interquartile range indicated that 50% of female custodians owned the landrace for a duration between 6.25 and

22 years. Among male seed custodians preserving the Bambara groundnut species, the mean ownership duration was 29.733 years, with a standard deviation of 20.869. The ownership periods ranged from 3 to 80 years, and the quartile values indicated that 50% of male custodians owned the landrace for a duration between 20 and 39.50 years.

Sorrel traditional Landraces

Six traditional landraces of sorrel were identified in the 11 villages based on the naming criteria. Two landraces were identified based on calyx size: the short calyx landrace called bii and the long calyx landrace called wegda in the Mooré language. Two types of landraces were also identified based on plant colour: green and red. The green landrace is called bit-pelga in the Mooré language, which can be translated into English as "white sorrel." The green landrace is also known as Pagb bii in Mooré, indicating that this landrace is mainly grown by females. This name also reflects the neglected status of this morphotype while the red morphotype, grown by both males and females is overused. As for the red landrace, it is called bit-miougou in Mooré, which can be translated into English as "red sorrel". Within this landrace, two variants are distinguished, namely the dark red calyx and the reddish calyx with a green base. The term bito in Mooré is used to refer to sorrel in general.

For female seed custodians conserving the sorrel species, the mean conservation duration was 31.692 years, with a standard deviation of 15.755. The conservation periods ranged from 8 to 60 years, and the interquartile range indicated that 50% of female seed custodians conserved the landrace for a duration between 24 and 36 years. In contrast, there were only two male seed custodians preserving the sorrel species, and their conservation duration was consistently 8 years.

The average duration of ownership among female seed custodians conserving the sorrel species was 13.962 years, with a standard deviation of 8.770. The duration ranged from 1 to 30 years, and the interquartile range suggested that 50% of female custodians owned the landrace for a period between 5 and 20 years. In the case of male seed custodians preserving the sorrel species, there were only two participants. Their mean ownership duration was 27.500 years, with a standard deviation of 3.536. Both male seed custodians owned the landrace for the same duration, with quartile values ranging from 26.25 to 28.75 years.

These findings shed light on the duration of landrace ownership among seed custodians, disaggregated by gender and species. The data revealed variations in the length of ownership, which could be influenced by factors such as cultural practices, seed availability, and local traditions. Further investigation is necessary to explore the underlying reasons for these ownership duration patterns and their implications for seed conservation efforts in Burkina Faso. Understanding gender dynamics within seed custodian farming is essential for developing inclusive strategies to support sustainable agriculture and biodiversity conservation.

Exploring the intersections of religion, seed conservation techniques, seed replacement, exchange, and ownership of custodian farmers.

The study highlights the active participation of individuals from different religious backgrounds in seed conservation efforts, with Animists, Christians, and Muslims identified as the main religious groups among seed custodian farmers. Christians constitute the largest group, accounting for 49.3% of seed custodians, followed by Muslims at 44.9%. This finding emphasizes the promotion of biodiversity conservation regardless of religious affiliation.

Diverse techniques are employed by farmers for seed conservation. The predominant techniques reported include the use of jerry cans (60.9% of farmers), clay pots (15.9%), and sacks (21.7%). These techniques ensure sturdy and secure storage, safeguarding seeds against external factors like moisture and pests. Notably, sorrel seeds are carefully separated based on landraces before drying and conservation, with some calyxes mixed with the seeds for landrace recognition. Ash is often used in conjunction with various containers such as cans, plastic bags, and canaries for storage.

The chi-square test reveals a significant association between education level and seed conservation technique (P = 0.0002). This finding highlights the influence of education in determining the choice of conservation methods.

Farmers exhibit a proactive approach to ensure crop production and maintain varietal purity, as 72.5% of them reported replacing seeds in case of loss. Seed replacement practices contribute to the preservation of desired traits and yield stability. Additionally, 34.8% of farmers engage in seed exchange for free, which fosters social networks and promotes the conservation and exchange of local seed landraces.

Examining the age and duration of seed conservation among different genders and crop landraces reveals interesting insights. Female custodian farmers for sorrel have a mean age of 48.462 years, while male guardians have a mean age of 49.000 years. For Bambara groundnut, female custodian farmers have a higher mean age of 51.462 years compared to 59.800 years for males. The duration of seed conservation ranges from 8 to 60 years, exhibiting variations based on gender and crop landrace.

Although traditional or local seed landraces dominate cultivation practices, a significant proportion of farmers (42%) reported cultivating improved seed landraces. The adoption of improved seeds can enhance crop productivity, disease resistance, and agricultural sustainability. Factors influencing the adoption include seed availability, accessibility, knowledge dissemination, and farmer training programs. Interestingly, no significant difference is found between cultivating improved landraces and seed replacement in case of loss (p = 0.4), shedding light on the motivations and challenges associated with cultivating improved landraces.

Exploring the dynamics of seed exchange among allochthonous and autochthonous farmers reveals that there is no significant association between farmers' origin and the practice of seed exchange (p = 0.2748), providing valuable insights into seed exchange practices in different farming communities.

The duration of seed conservation does not significantly vary based on the species (P = 0.119) or the conservation technique (P = 0.15). However, a significant difference is observed in the duration of conservation based on gender (p = 3.451 x 10-5), even when analysed within each species (within sorrel: P = 0.018; within voandzou: P = 0.002). This gender-based difference highlights the role of gender in seed conservation practices.

CONCLUSIONS

The present study highlights the crucial role of custodian farmers in Burkina Faso in preserving and conserving neglected and underutilized crops, particularly focusing on Bambara groundnut (*Vigna subterranea*) and sorrel (Hibiscus sabdariffa). Female farmers were found to be the majority of identified custodians, actively participating in seed conservation initiatives through various techniques such as cooking pots, canaries, and ash. Seed exchange is also a common practice among them, supporting crop diversity and social cohesion within local communities. The discovery of multiple landraces held by custodian farmers across various villages emphasizes the need for further research into understanding factors influencing the distribution and perpetuation of traditional landraces and methods employed for their conservation. Effective conservation strategies that engage local communities are paramount for ensuring global food security. Future scientific pursuits should prioritize developing inclusive and sustainable approaches that provide unequivocal support to custodian farmers, facilitating the preservation of traditional crop varieties. Supporting and acknowledging the knowledge and practices of custodian farmers in the realms of seed conservation and crop diversity is essential for securing the future of agriculture not only in Burkina Faso but also globally.

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HUMUS QUALITY OF ANTHROPOGENIC SOILS FROM FLYSCH DEPOSITS. CASE STUDY: KAŠTELA BAY, CROATIA

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ABSTRACT

The study aimed to evaluate the humus quality of anthropogenic soils derived from flysch deposits. A total of 10 samples were collected in the top-soil horizon (0-20 cm) of olive groves in Kaštela Bay in Middle Dalmatia, Croatia. Five samples were taken from traditional low-input olive groves (TOG), and the other five samples were from intensive ones (IOG). The soil samples were analysed for basic soil properties: particle size distribution, pH, carbonate content, P_2O5, K_2O, and soil organic carbon (SOC) content. Humic substance isolation was done following the procedure given by Schnitzer (1982). Spectroscopic characterization of humic substances was carried out by measuring absorbances in VIS spectral range (400-700 nm). Optical index E_4/E_6 (ratio of optical absorbance at 465 and 665 nm for humic substances in solution) was calculated. The mean value of SOC content in soils of TOG was lower than in soils under IOG (1.20 and 1.60%, respectively). The absorption spectrum of the studied soils showed a monotonous decrease of absorbance from 400 to 700 nm. The E_4/E_6 indices for soils under TOG varied from 4.60 to 5.00, while for soils under IOG were in the range of 4.83 – 6.89. The E_4/E_6 indices > 4 revealed low humus quality in studied soils. However, soils under TOG have lower mean values E_4/E_6 index in comparison to soils under IOG (4.76 and 5.70, respectively), indicating slightly higher humus quality.

Key words: *E_4/E_6 index, olive groves, VIS spectroscopy.*

INTRODUCTION

Soil organic matter supports multiple soil ecosystem functions underpinned by processes such as C sequestration, N mineralization, aggregation, promotion of plant health and compound retention (Hoffland et al., 2020.) It is a heterogeneous mixture of organic compounds that differ in stability, decomposition degree, and turnover rate (Huang et al., 2008). Humic substances are generally classified into humic acid, fulvic acid, and humin based on their solubility in water as a function of pH (MacCharthy et al., 1990). Although numerous methods have been developed for isolation and quantification of humic substances (van Zomeren and Comans, 2007), method of Schnitzer (1982) is the most widely used. It is

successful in maximizing extraction of humic compounds and minimizing their degradation, and suitable for all soil types regardless of carbonate content.
Numerous non-destructive spectroscopic techniques (UV–Vis, DRIFT, SFS, FTIR, and ^{13}C-NMR) provide valuable information on humic substance structure and properties (Chen et al., 2002). Since humic substances show strong absorption in the UV-Vis range (190-700 nm) due to the presence of aromatic chromophores (Schnitzer and Khan, 1975) the UV-Vis spectroscopy is one of the most frequently used methods for humic substance characterization (Shirshova et al., 2006). The E_4/E_6 index calculated as the ratio of optical absorbance at 465 to 665 nm for humic substances in solution is commonly used to evaluate humus quality (Aranda et al., 2011; Reddy et al., 2014; Bensa et al., 2016). It is negatively correlated to the content of condensed aromatic structures (Stevenson, 1994) and decreases as condensation, aromaticity, and molecular weight of humic substances increase.
The impermeable or poorly permeable flysch deposits are especially prone to erosion by torrential watercourses (Pollak et al., 2010) which increases the loss of organic matter from top-soil. Therefore, many authors reported low humus content in soils derived from flysch deposits (Miloš and Maleš, 1998; Bogunović et al., 2009; Aranda et al., 2011). However, restricted data are available on humus composition and quality in these soils. In addition, anthropogenic activities such as tillage, fertilization, and the use of pesticides affect humus quality in agricultural soils.
The aim of the paper is: (i) spectroscopic characterization of humic substances and (ii) evaluation of humus quality of anthropogenic soils derived from flysch deposits under traditional low-input and intensive olive groves.

MATERIAL AND METHODS

The study was conducted in the olive growing area of Kaštela Bay in Midldle Dalmatia, Croatia, Figure 1a. The study area is characterized by a Mediterranean climate (Csa) with hot and dry summers and mild rainy winters (Filipčić, 1998). Kaštela Bay coastal area is built of Eocene Flysch marls, sandstones, and siltstones with lenses of calcirudites and calcarenites (Marinčić et al., 1971). Impermeable clastic flysch deposits and sloping terrain (mainly 10-30%) make this area vulnerable to erosion. Soils of the study area have an alkaline reaction, high carbonate content, very low to medium humus content, and silty loam texture (Miloš and Maleš, 1998). According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015) studied soils can be classified as Regosols (Calcaric, Siltic/Loamic, Escalic). The olive growing in the study area characterizes dry farming, including traditional low-input and intensive growing systems.
A total of 10 top-soil samples (0-20) cm were collected as composite samples. The five samples were taken in the olive groves under the traditional low-input system, and the other five from the olive groves with the more intensive growing system, Figure 1b. Soils in the traditional olive groves (TOG) are rarely tilled, fertilized, and treated with pesticides. The intensive olive growing system (IOG) includes

more intense fertilization (mineral and organic), plant protection, and regular soil tillage.

Figure 1 Location of the study area (A) and soil sampling sites (B). Locations of traditional olive groves are symbolised by yellow triangles and intensive ones by red circles

The disturbed soil samples were prepared for analysis according ISO 11464:2006. Basic soil analyses included: particle size distribution (ISO 11277:2009), pH (ISO 10390:2005), carbonate content (ISO 10693:1995), P_2O_5 and K_2O (Egner et al., 1960) and humus content (method of Tjurin, JDPZ, 1966). Soil organic carbon (SOC) content was calculated by dividing the humus content with the Van Bemmelen factor (1.724). The isolation of soil humic substances (HS) was made by the Schnitzer method (1982). The VIS spectra were measured by Shimadzu UV 1700 spectrometer in the range of 400-700 nm. The extracts were prepared as a mixture of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$.

RESULTS AND DISCUSSION

Alkaline soil reaction (pH_{KCl} >7.2) was established in the soils under both types of olive production with minor differences, Table 1. The studied soils were highly calcareous (mean values of $CaCO_3$ in soils under TOG and IOG 58.6 and 47.9%, respectively). The SOC content was low in the soils under TOG (0.90-1.60%) and low to medium in the soils under IOG (1.09-2.12%), Table 1. The mean value of P_2O_5 in the soils under TOG (7.1 mg/100 g of soil) pointed to a poor supply with P_2O_5, with high variation (CV 45.5). The soils under IOG were moderately supplied with P_2O_5 (mean value 14.8 mg/100 g of soil), Table 1. Even more pronounced differences were observed for K_2O concentrations. The soils under TOG contained medium K_2O content (mean value 16.4 mg/100 g of soil), while the soils under IOG were richly supplied with K_2O (mean value 48.1 mg/100 g of soil), Table 1. It can be attributed to mineral fertilization applied in higher doses to soils under IOG.

Table 1 Descriptive statistic for basic chemical properties of studied soils

	pH_{H2O}		pH_{KCl}		$CaCO_3$ %		SOC %		P_2O_5 mg/100 g of soil		K_2O	
	TOG	IOG	TOG	IOG	TOG	IOG	TOG	IOG	TOG	IOG	TOG	IOG
Min	8.35	8.28	7.48	7.23	56.5	31.6	0.90	1.09	4.6	11.3	12.8	34.5
Max	8.50	8.54	7.72	7.58	64.1	61.6	1.60	2.12	13.4	18.1	19.2	73.5
Mean	8.43	8.40	7.59	7.48	58.6	47.9	1.20	1.60	7.1	14.8	16.4	48.1
Med	8.45	8.39	7.59	7.54	56.6	50.5	1.16	1.68	5.8	15.1	17.0	47.4
SD	0.06	0.09	0.08	0.13	0.23	10.1	0.23	0.34	3.21	2.41	2.09	13.9
CV	0.75	1.1	1.0	1.8	5.0	21.1	19.3	21.5	45.5	16.3	12.8	28.9

Min – minimum; Max maximum; Med- median; SD- standard deviation; CV coefficient of variation

Particle size distribution analysis revealed the domination of silt particles for soils under both management types. However, soils under IOG have a higher content of clay particles in comparison to soils under TOG (mean values 24.24 and 15.72%, respectively). The texture classes of soils under TOG were loam and silty loam, while soils under IOG were silty loams and clay loams, Figure 2.

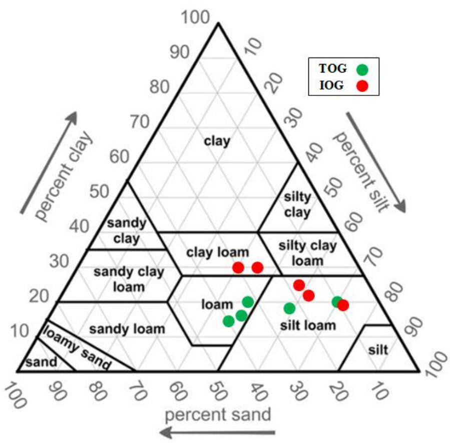

Figure 2 Distribution of soil samples on texture triangle (FAO/UNESCO, 1990)

The established properties of studied soils correspond to literature findings for soils on flysch deposits in Middle Dalmatia (Miloš and Maleš 1998; Bogunović et al., 2009; Miloš and Bensa, 2019).
Humic substances showed strong absorption in the UV-Vis range (Schnitzer and Khan, 1975). UV-Vis spectra of humic substances can characterize their quality, maturity, and condensation degree (Fuentes et al., 2006). The UV-Vis spectral lines of humic substances extracted from studied soils are shown in Figure 3. In general, lower absorbances and a milder decline in the spectral curves were observed for the soils under TOG compared to IOG. It implies the domination of aromatic over aliphatic structures (Pospíšilová and Fasurová, 2010; Fasurová and Pospíšilová, 2010) in the soils under TOG.

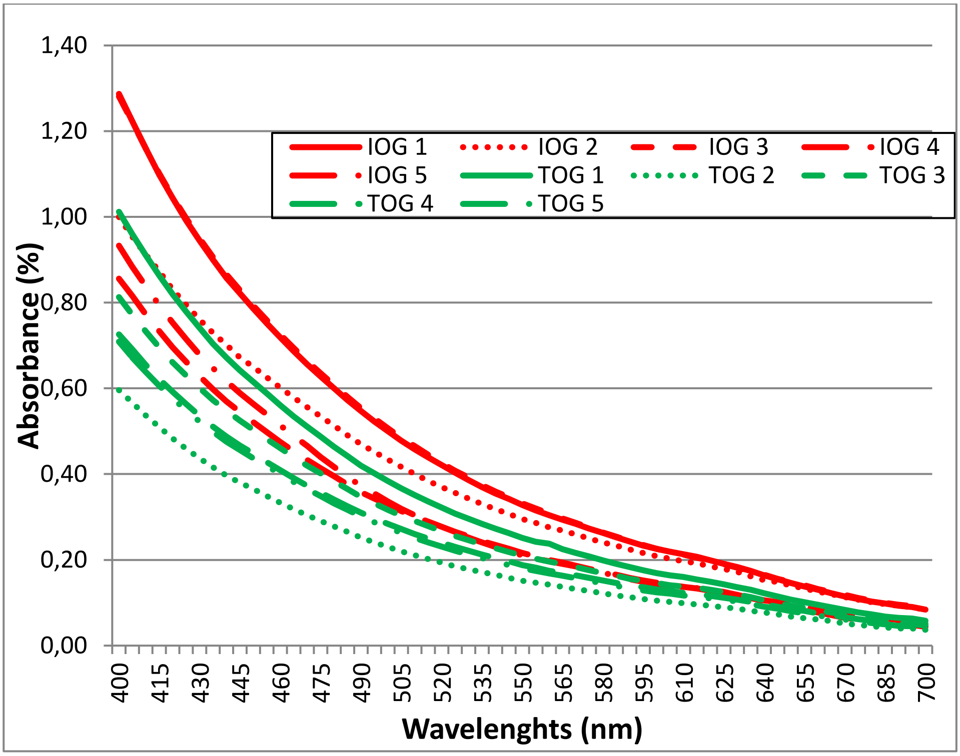

Figure 3 UV-Vis spectra of exctracted humic substances in the studied soils

The absorbance of humic substances at the wavelength of 465 nm is associated with the first phase of the humification process (fulvic acids), while absorption at 665 nm is related to well-humified substances (humic acids) (Stevenson, 1994). Therefore, the calculated optical index E_4/E_6 (ratio of optical absorbance at 465 to 665 nm for humic substances in solution) is negatively correlated to the content of condensed aromatic structures (Stevenson, 1994; Fuentes et al., 2006). It decreases as condensation, aromaticity, and molecular weight of humic substances increase. The E_4/E_6 indices of humic substances isolated from studied soils are shown in Table 2.

Table 2 The E_4/E_6 indices of humic substances isolated from the studied soils

Management type	Soil sample number					Mean value
	1	2	3	4	5	
TOG	5.00	4.68	4.73	4.60	4.78	4.76
IOG	5.63	4.83	5.55	5.59	6.89	5.70

In soils under TOG E_4/E_6 indices were 4.60 to 5.00, with mean value of 4.76. Soils under IOG had E_4/E_6 indices in the range of 4.83 – 6.89, with mean value of 5.70. The E_4/E_6 indices above 4 in all studied soils indicated low humus quality (Chen et al., 2022; Pospišilova et al., 2010; Reddy et al., 2014). However, a narrower range and lower mean value of E_4/E_6 in soils under TOG (Table 2) indicated slightly higher and a more uniform humus quality. It can be ascribed to a less soil cultivation since tillage is unfavourable for humification and the formation of stable humic substances. It is consistent with the study of Murage and Voroney (2008) and Seddaiu et al. (2013), who also found higher humus quality in less tilled soils. Aranda et al. (2011) reported similar E_4/E_6 indices in soils under olive groves derived on marls in Spain (4.37-5.15). Authors established lower values (higher humus quality) in soils under organic farming compared to conventional olive production.

CONCLUSIONS

Spectroscopic characterization of humic substances isolated from the anthropogenic soils derived on flysch deposits under olive groves revealed domination of aliphatic over aromatic structures. In general, studied soils have low humus quality. However, lower E_4/E_6 indices in soils under traditional low-input olive groves indicate slightly higher humus quality. Therefore, it can be concluded that agricultural practices in intensive olive production (mainly tillage) negatively affected humus quality. The results of the study point to the need for improvement of humus quality (and content) in anthropogenic soils on flysch deposits under olive groves in fragile Mediterranean environment.

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DETERMINATION OF SALMONELLA ANTIBODY TITERS IN THE MEAT OF HEALTHY AND PNEUMONIA DISEASED PIGS

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ABSTRACT

Salmonella is the most common pathogenic microorganism found throughout the environment and common source of bacterial foodborne-related illness. A practical tool for detecting *Salmonella* infection in slaughtered pigs is the serological examination of meat juice. *Salmonella* was determined in meat juices from healthy and pneumonia diseased pigs. *Salmonella* antibodies in pig meat juice were determined by a screening immunoenzymatic analysis method using the IDEXX HerdChek Swine *Salmonella* Test Kit (IDEXX Laboratories, Switzerland). The samples were evaluated according to the optical density ratio of the test sample and the positive control sample. Meat samples from diaphragm pillars were taken from 277 pigs at slaughter house. A total of 63 samples were serologically positive from all tested pig meat juice samples (22.74 %) (CI95 % 18.2 – 28.0 %). One positive sample was found in the meat juice of healthy pigs, while 25 and 37 samples were found to be seropositive in the diseased pig groups, with S/P of 0.76 ($p < 0.001$) and 0.91 ($p < 0.001$) respectively. In pigs with pathological lung changes 35.23 % seropositive samples of all diseased pigs were found. S/P ratio in control group was 0.08 while in pig with different lung lesions meat juice samples S/P ration reached 0.25 and 0.32 respectively ($p < 0.001$). The lowest S/P value in meat juice samples were found in pigs with moderate lung lesions and were found in 56.88 % meat juice samples, while in pigs with intensive lung lesions meat juice samples S/P ratio was 58.13 % ($p < 0.001$). The highest S/P value was found in pigs with intensive lung lesions and reached 0.77 % of all tested samples.

Keywords: *Salmonella, pigs, meat juice, antibody detection.*

INTRODUCTION

Salmonella is one of the most common pathogenic microorganisms found throughout the environment. The pathogenicity of individual serotypes is usually host-specific, such as *Salmonella dublin* is common in cattle, *Salmonella choleraesuis* in pigs, or *Salmonella typhi* in humans. Most of the known serotypes, such as *Salmonella typhimurium* and *Salmonella enteritidis* are not adapted to their specific hosts and therefore can infect many animal species, including humans (Nielsen *et al*., 1995; Evangelopoulou *et al*., 2014; Bonardi, 2017; Sun *et al*., 2020; D'Incau *et al*., 2021).

Salmonella transmission between hosts occur by air or direct contact. Weaned piglets are most often infected with *Salmonella*. It has been found, that *Salmonella typhimurium* can cause disease of young piglets, 6-12 weeks of age, and in some cases of adult pigs, although *Salmonella choleraesuis* can cause salmonellosis in pigs of all ages (Wilcock and Schwartz, 1992; Savic *et al*., 2021). *Salmonella* infection is not uncommon in suckling piglets, although it is extremely rare due to maternal immunity (Gray and Fedorka-Cray *et al*., 2000; Matiasovic *et al*., 2013). Clinically, two porcine salmonellosis syndromes are distinguished, one involving *Salmonella typhimurium* infection with enterocolitis and the other with *Salmonella choleraesuis* infection with septicemia (Wilcock and Schwartz, 1992; Savic *et al*., 2021).

Pigs can be infected with *Salmonella* in their housing, by air during transportation from other diseased pigs or by direct contact from a *Salmonella* contaminated environment (Swanenburg *et al*., 2001; Arguello *et al*., 2013). *Salmonella* is usually isolated from the tonsils, intestinal lymph nodes and intestinal contents of diseased pigs (Proux *et al*., 2000; Swanenburg *et al*., 2001; Novak *et al*., 2007; Deane *et al*., 2022).

Slaughtered pigs, infected with *Salmonella* but showing no signs of the disease, may be the most important source of *Salmonella typhimurium* infection, which poses a threat to the entire food chain. Despite the fact that, based on the consistent application of *post-mortem* inspection rules, classical zoonoses have been largely eliminated and the health of slaughtered animals has improved in recent decades, and the groups of slaughtered animals are more homogeneous, the problem of consumer safety when supplying meat from slaughtered pigs for food has not yet been resolved. Zoonoses occurring during this period are more subclinical, there are no visible changes in carcasses and organs, so, apart from obvious pathological changes, zoonoses in slaughtered pigs are the main cause of diarrhea (food poisoning) in humans (Bonardi, 2017). Slaughtered infected pigs without clinical signs can be the most important source of *Salmonella typhimurium*, which is very dangerous for the entire food chain. The development of effective, inexpensive and rapid diagnostic methods is one of the factors that reduce salmonellosis outbreaks in humans and animals. Since 1993 in Denmark, enzyme-linked immunosorbent assay (ELISA) has been introduced in salmonellosis monitoring studies. The tests were carried out using blood serum from pigs, and meat juice was used for slaughtering pigs (Nielsen, 1998; Mousing *et al*., 1997; Bak and Sørensen, 2006).

ELISA in experimental studies and routine diagnostics shows important advantages - specificity, sensitivity and easy application (Gray *et al.*, 1999; Nowak *et al.*, 2007). Considering the fact that antibody titers in meat juice are slightly lower compared to blood serum, the advantage of meat juice testing by changing the sample dilution ratio has been proven, especially since it is inexpensive and relatively fast compared to bacteriological test methods (Proux *et al.*, 2000; Viana *et al.*, 2020).

MATERIALS AND METHODS

Selection and grouping of pigs for study

For the detailed analysis of lung diseases in pigs, detected during *post-mortem* inspection, one meat company was selected, to which pigs raised in different regions of Lithuania were transported for slaughter. For further research, pigs were reared under the same conditions of rearing and storage and transported to the slaughterhouse at a distance of 65 km. After slaughtering, the internal organs of the pigs were examined and the macroscopic pathological changes of the lungs, heart and liver were registered. Based on the methodology for the assessment of lung pathological changes proposed by Blaha and Neubrand (1994), according to the determined data of lung changes, groups of pigs were formed: pigs with moderate pneumonia (11-30 % of lungs affected) with signs of mild pleuritis (group Pn 2 + Pl 2) and pigs with intensive pneumonia (over 30% of lungs affected) with pleuritis, covering a large area of the lungs (group Pn 3 + Pl 3). It should be noted that all lung changes identified had signs of a chronic type. The control group consisted of pigs that did not show any pathological changes during post-*mortem* examination.

Collection of muscle samples and extraction of meat juice

At the slaughterhouse after 1 hour after slaughter *musculus pillar diaphragmaticus* in size of 3.0 x 1.0 x 1.0 cm were collected from slaughter pigs. The muscle samples were placed in 50 ml plastic bags and transported to the laboratory immediately after collection. In total, muscles were collected from 277 pigs. Muscles were frozen at 220 °C**.** 1 day before the beginning of the study, the investigated meat samples were transferred to a refrigerator at a temperature of +4 **°C** and kept for 24 hours. During thawing, meat juice was released from the muscles and collected in the bottom of the containers.

Detection of *Salmonella* antibodies in meat juice samples

Antibodies against *Salmonella* spp. in pig meat juices were determined by the overview method of immunoenzymatic analysis. A commercial diagnostic kit (IDEXX HerdChek Swine *Salmonella*, IDEXX Laboratories, Switzerland) was used for this purpose. Analysis was performed consistently according to the diagnostic kit manufacturer's methodology. Each of the 96 wells of the IFA plate was coated with antigens (serogroups B, C1, D). Before the study, the meat juice of the test pigs was diluted in a ratio of 1:2. Diluted meat juice samples were transferred into the wells of the plastic plate intended for analysis with a multichannel pipette, 100 µl each. Undiluted negative and positive control serum samples of 100 µl each were also added to the wells of the plate intended for them. The samples were incubated for 30 min. at a temperature of 25 **°C**. After incubation, the wells of the plate were washed with a buffer solution of 300 µl to each well of the plate. The procedure is repeated 5 times for 2 minutes. After incubation, the plate was coated with 100 µl of specific immunoglobulin conjugated with horseradish peroxidase. Incubated for 30 min. at a temperature of 25 **°C**. The plate was washed again with buffer solution 300 µl to each well of the plate, repeating the procedure 5 times. After washing, 100 µl of tetramethylbenzidine substrate was added to each well of the plate and incubated for 15 min. at room temperature. The reaction was stopped by adding 100 µl stop reagent (hydrochloric acid) to the wells of the microplate.

The intensity of the color is directly proportional to the amount of antibodies in the test samples. The results of the research were evaluated by spectrophotometer by measuring the optical density (OD) of the test sample at a wavelength of $\lambda = 650$ nm. The difference between the means of the positive and negative control sera had to be greater than or equal to 0.150 when evaluating the results of the assay. The mean of the negative control serum had to be less than or equal to 0.200. The OD of the samples was compared with the positive control OD of the buffer solution. Samples are considered positive if their OD is 10 percent or more high than the OD value of the buffer positive control or ratio of optical density of the test sample and the positive control sample $S/P \geq 0.25$. Samples are negative if their OD values are less than 10% buffer control OD or $S/P < 0.25$. To determine the reliability of the test results, the ELISA was repeated 2 times (S/P-1, S/P-2).

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics Version 27. Differences in the test properties of the compared groups are expressed as means and RMSE (root mean square errors). The differences between the investigated groups were evaluated using Fisher's LSD criterion (α=5%). The differences were considered to be statistically significant when $p< 0.05$.

CI was calculated by the Wilson method.

RESULTS AND DISCUSSION

Meat juices from 277 slaughtered pigs were tested for *Salmonella* specific antibodies. Evaluating the results of the analysis of pig meat juice using the ELISA method, it was found that 63 pig meat juice samples (22.74 %) were serologically positive of all tested samples (CI 95 % 18.2 – 28.0 %). Analyzing *Salmonella* antibody titers, the number of seropositive pigs detected at *post-mortem* examination was unevenly distributed according to the degree of lung damage in pigs. *Salmonella* antibodies were found in only one sample of all tested meat juice samples of the control group of pigs, while 35.23 % of seropositive samples were found in the meat juice samples of pigs with pathological lung changes of all samples from diseased pigs. 22.90 % of pigs with moderate lung lesions meat juice

samples were serologically positive, and 28.70 % with intensive lung lesions meat juice samples were serologically positive.
To determine the reliability of the test results, the ELISA was repeated 2 times. The results of ELISA tests are presented in Table 1, where S/P-1 is the result determined for the first time, and S/P-2 is the result for the second time. S/P was 0.08 ($p < 0.001$) in meat juice from control pigs, while S/P in meat juice from diseased pigs was greater than 0.25 of all tested samples. S/P in meat samples of pigs with intensive lung lesions was 0.32 ($p < 0.001$). The results obtained in both study periods did not differ and confirmed their reliability.

Table 1. Results of the *Salmonella* specific antibody test in the tested meat juice samples.

	Control group; n = 38	Pn 2 + Pl 2; n = 109	Pn 3 + Pl 3; n = 129
S/P–1	0.08 ± 0.008^{a}	0.26 ± 0.041^{b}	0.32 ± 0.047^{b}
S/P–2	0.08 ± 0.006^{a}	0.25 ± 0.041^{b}	0.32 ± 0.05^{b}
a, b – $p < 0.001$			

In the evaluation of pig lung pathologies and *Salmonella* antibody titers in meat juice, it was found that one sample of the control group was serologically positive, while 25 and 37 samples were found to be seropositive in the diseased pig groups, with S/P of 0.76 ($p < 0.001$) and 0.91 ($p < 0.001$) respectively (Table 2).

Table 2. Effect of lung lesions on *Salmonella* antibody test results.

Group	Result	n	S/P–1	S/P–2
Control group	Seronegative	38	0.08 ± 0.006	0.07 ± 0.003
	Seropositive	1	0.29	0.29
Pn 2 + Pl 2	Seronegative	84	0.12 ± 0.024^{a}	0.11 ± 0.024^{a}
	Seropositive	25	0.76 ± 0.112^{b}	0.71 ± 0.119^{b}
Pn 3 + Pl 3	Seronegative	92	0.08 ± 0.003^{a}	0.07 ± 0.003^{a}
	Seropositive	37	0.91 ± 0.116^{b}	0.93 ± 0.127^{b}
Pn 2 + Pl 2 Pn 3 + Pl 3	Seronegative	214	0.09 ± 0.009^{a}	0.09 ± 0.01^{a}
	Seropositive	63	0.84 ± 0.082^{b}	0.83 ± 0.089^{b}
a, b – $p < 0.001$				

When evaluating the distribution of S/P in the meat juice samples of healthy and diseased pigs, it was found that the lowest S/P value (0.10) was in 79.48 % meat samples of the control group of all tested samples and only 5.12 % amounted to the highest level (0.30). The lowest S/P value of diseased pigs was 57.56 % while the highest value was 3.00 and reached 0.42 % (Fig. 1).

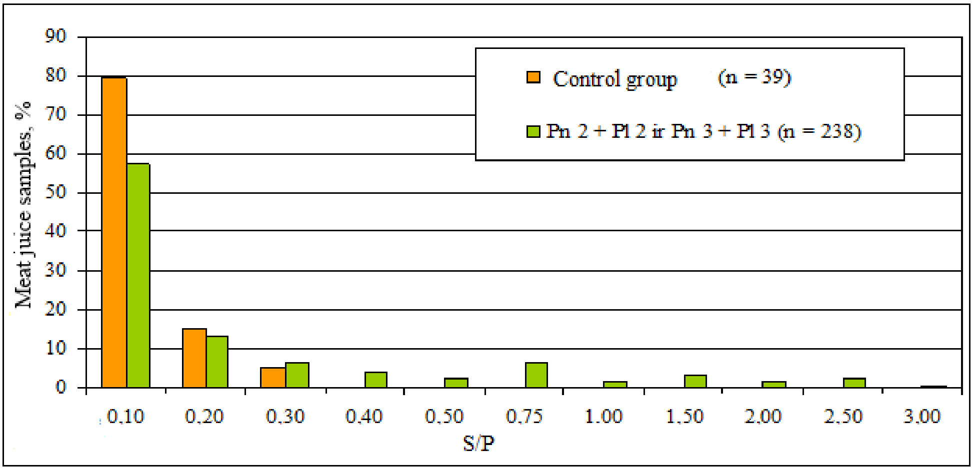

Fig. 1. Optical density ratio (S/P) of *Salmonella* specific antibodies in meat juice of healthy and diseased pigs.

When analyzing the distribution of *Salmonella* antibodies S/P values according to the degree of lung damage, the values of Pn 2 + Pl 2 and Pn 3 + Pl 3 groups were almost evenly distributed - the lowest S/P value in meat juice was found in pigs with moderate lung lesions and was found in 56.88 % meat juice samples, while in pigs with intensive lung lesions meat juice samples S/P ratio was 58.13 %. In addition, the highest value (3.00) was found in pigs with intensive lung lesions and reached 0.77 % of all tested meat juice samples in this group. The highest S/P value of Pn 2 + Pl 2 group samples was 2.5 and reached to 1.83 % of all tested meat juice samples in this group (Fig. 2).

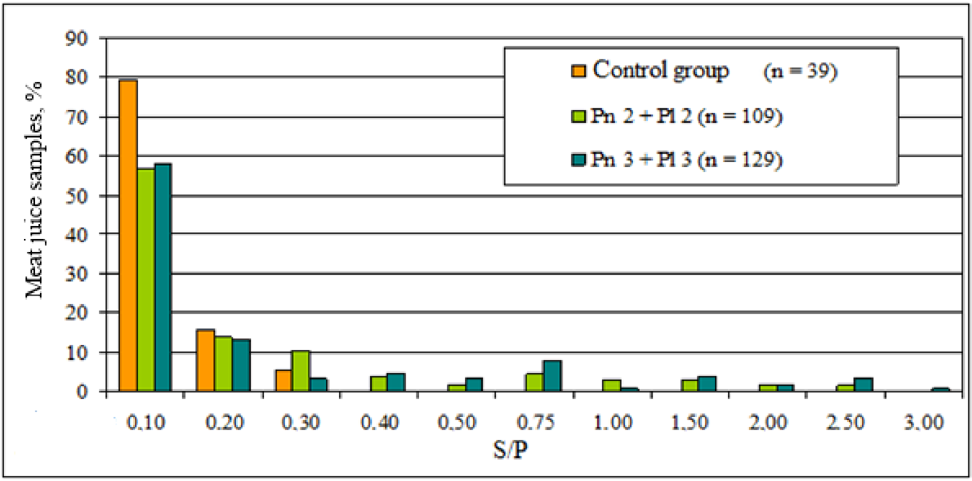

Fig. 2. Optical density ratio (S/P) of *Salmonella* specific antibodies in meat juices of different pig groups.

Blood serum is mostly used in biochemical and serological tests. The use of blood serum in routine *post-mortem* examination is difficult due to the speed of work in the slaughter line and the too short time for taking blood for examination. Therefore, in the search for newer and better test methods to improve the quality of *post-mortem* inspection, the suitability of meat juices has been proven due to their simple use and the possibility of automating the test process itself (Nielsen, 1998). The collection of blood samples on the farm as part of the ante-mortem inspection can complicate the slaughter process due to the labor and time costs of sampling. And the method of taking meat samples after slaughtering the animal turned out to be superior also because it did not require specific technical knowledge. In addition, the testing used to identify the health status of pigs can be not only improved but also simplified by implementing centralized testing systems (Le Potier *et al*., 1998). Pig salmonellosis can cause not only economic losses, but also a lot of damage to human health, so very fast, accurate and sensitive methods for S*almonella* detection are needed. Routine salmonellosis testing introduced in Denmark has proven to be effective in reducing salmonellosis outbreaks in the country (Wegener *et al*., 2003). Our immunoenzymatic analysis using meat juices suggested that this ELISA method could be applied as a rapid method for *Salmonella* spp. as a routine *post-mortem* monitoring method. It should also be noted that the ELISA method only confirmed positive samples in the meat juice at the genus level, but did not identify the species or serotype. On the other hand, identification of the species or serotype of *Salmonella* is not very important for epidemiological studies or monitoring purposes, especially in pig farms where *Salmonella* infection is very common and where more than one *Salmonella* serotype has been isolated (Feder *et al*., 2001; Novak *et al*., 2007; Edel *et al*., 2023).

CONCLUSION

The results of our study showed that 22.74 % of all tested samples of pig meat juice were serologically positive. The number of seropositive pigs was unevenly distributed according to the degree of lung damage in the pigs determined at *post-mortem* examination. *Salmonella* antibodies were found in only one sample of all tested meat juice samples of the control group of pigs, while 35.23 % of seropositive samples were found in the meat juice samples of pigs with pathological lung changes of all samples from diseased pigs. 22.90 % were serologically positive for *Salmonella* in meat juice samples of pigs with moderate lung damage, and 28.70 % of pigs with severe lung damage.

The obtained results allow this ELISA method to be applied to the control of food products, when it is necessary to check the suspicious raw material very quickly, within one day.

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BUSINESS MODELS IN THE CIRCULAR ECONOMY

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ABSTRACT

During the recent decades there is an unprecedented growth in the demand for resources which has triggered a vast interest in shifting to a circular model of production. The classic business model, in which the production of new goods and the provision of services is reduced to the continuous use of new material resources, does not correspond to the views and concepts of ecological business. A new business model should be implemented. A circular economy is an economic model that aims to reduce waste and maximize the use of resources. In a circular economy, products and materials are reused, repaired, or recycled rather than being disposed of after use. The goal is to create a closed-loop system where resources are continuously circulated and waste is minimized. This concept is in contrast to the traditional linear economy, where products are made, used, and then discarded as waste. In a circular economy, products are designed with durability and recyclability in mind, and materials are kept in use for as long as possible. The circular economy has the potential to increase the resilience to the physical effects of climate change. Its principles offer businesses opportunities for innovation that will lead to reducing materials costs, increasing asset utilisation, and responding to changing customer demands. By addressing both the causes and effects of climate change, the circular economy can help meet The UN Sustainable Development Goals and lead to improved air quality, reduced water contamination, and better protected biodiversity.

Keywords: *circular economy, circular business models, sustainable development, EU.*

INTRODUCTION

Natural resources and the materials derived from them represent the physical basis for the whole economic system (OECD, 2019). During the recent decades there is an unprecedented growth in the demand for resources, so this has triggered a vast interest in shifting to a more resource efficient and circular model of production. In order for the environmental benefits to be achieved, the policy frameworks need to create the conditions for a wider implementation of circular business models and to put efforts to stop occupying only small economic niches, but to be introduced to and integrated in all economic activities.

The current prevailing economic design has its roots in the historically unequal distribution of wealth by geographic region (Ellen MacArthur Foundation, 2013). Resource users were largely concentrated in the most developed regions, and industrial nations possessed abundant material resources and energy. With this distribution, materials were cheaper than human labor and so, the implemented business models relied on extensive use of materials and saved on human labor. As a result is the neglect of recycling and reuse and an increase in waste, and, as well, a huge waste of resources and inefficiency. But in the new millennium the linear economy models are no longer successful as the depletion of resources, the cost of materials and the increasing prices of products are some of the main reasons for the companies to lose their competitive advantages and to be compelled to shift to more sustainable models.

There are a vast number of definitions of circular economy (CE) (Kirchherr et. al., 2017) and yet it is most often related to sustainable development, economic prosperity and environmental quality, while its impact on social equity and future generations is not fully taken into account. The CE is usually represented as a combination of activities related to reducing, reusing and recycling models but the need of overall and systematic transition towards it, as well as the need of introducing new business models or relating the consumers with it, are neglected. CE systems (COM (2014) 398 final 2) preserve added value in products for longer and eliminate waste by keeping resources within the economy. The transition to a more CE requires changes all along the value chains and a complete systemic change and innovation not only in technology, but also in organization, society, funding methods and policies. Even in a highly CE, some element of linearity will remain as fresh resources are needed and residual waste is disposed of.

Adaptation is a leading component of the global response to climate change (COM(2021) 82 final). But the EU and the global community are still underprepared for the increasing intensity, frequency and pervasiveness of climate change impacts, especially as emissions continue to rise. As a response to climate change, the new EU Adaptation Strategy paves the way for achieving climate neutrality in 2050. But in order to do so (COM (2020) 98 final), the process of shifting towards circular models and reduction of greenhouse gas emissions should be speeded up. The main efforts to manage climate change have been focused on a transition to renewable energy (Ellen MacArthur Foundation, 2021). Though crucial and in line with the CE principles, these measures can only address 55% of emissions. So it is of great importance the remaining 45% to be addressed as well. Systemic change of energy and industrial systems, land management, buildings, and infrastructure will be needed to reach net-zero emissions by 2050 and limit global warming to 1.5˚C. By addressing both the causes and effects of climate change, the CE can help meet The UN Sustainable Development Goals and lead to improved air quality, reduced water contamination, and better protected biodiversity.

In the New Circular Economy Action Plan (COM (2020) 98 final) seven sectors with a huge potential and expected great effect on establishing a well working CE model are defined - electronics and ICT, batteries and vehicles, packaging, plastics, textiles, construction and buildings, food, water and nutrients.

The main objective of this paper is to outline the so far introduced CE models and the impact and benefits arising from their implementation.

MATERIALS AND METHODS

The paper is based on a comprehensive review of research papers, reports, action plans, programs and data related to CE, its implementation and the introduction of circular business models that are to support the transition towards a CE and sustainable growth at EU level.

Different scholars (Kirchherr, 2021; Lahti et al., 2018; Lieder and Rashid, 2016; etc.) identify following key elements that contribute to the effectiveness of the circular economy: **(1) Design for durability and recyclability**: Products are designed to be long-lasting, easy to repair, and made from materials that can be recycled or reused at the end of their life cycle. This promotes resource efficiency and reduces waste; **(2) Resource efficiency**: The CE emphasizes the efficient use of resources by reducing waste and maximizing the value extracted from materials. This includes strategies such as reusing and repurposing materials, implementing recycling programs, and optimizing production processes; **(3) Closing the loop**: In a CE, materials and products are kept in circulation for as long as possible. This involves strategies such as recycling, remanufacturing, and refurbishing to extend the lifespan of products and prevent them from becoming waste; **(4) Collaboration and innovation**: The CE requires collaboration between different stakeholders, including businesses, governments, and consumers. It also encourages innovation in areas such as product design, recycling technologies, and business models to drive the transition towards a CE; **(5) Sustainable consumption and production**: The CE promotes a shift towards more sustainable consumption patterns, such as sharing and collaborative consumption, as well as responsible production practices that minimize environmental impact; and **(6) Economic incentives and policy support**: Governments can play a crucial role in facilitating the transition to a CE by implementing policies and regulations that incentivize sustainable practices. This can include measures such as tax incentives for recycling, support for research and development of CE technologies, and promoting sustainable procurement practices. By incorporating these elements, the CE aims to create a more sustainable and resilient economic system that minimizes waste, reduces environmental impact, and maximizes resource efficiency.

In the Impact Assessment Report of The new EU Strategy on Adaptation to Climate Change (SWD (2021) 25 final) the policy options, and the corresponding actions and measures, considered for inclusion in the new Strategy are represented. The first option is based on the 2013 Strategy and its eight actions while the second includes six additional actions. The assessment points out that the second option is more preferable because it adds greater ambition to the Union’s actions on climate change adaptation, in particular with regard to international actions. Thus, additional and more extensive measures are needed for better adaptation to climate change.

Politics at the international level must become the main driver for achieving the goals of the CE (C. F. A. Arranz et. al., 2023), as their successful implementation requires a new vision and rethinking of existing models. These changes cannot be introduced by business itself, and therefore substantial support is needed at the level of policy making and strategy development to overcome the obstacles to achieving a CE in existing economic and industrial systems.

RESULTS AND DISCUSSION

The CE has the potential to increase the resilience to the physical effects of climate change (Ellen MacArthur Foundation, 2021). Its principles offer businesses opportunities for innovation that will lead to reducing materials costs, increasing asset utilisation, and responding to changing customer demands. Together, these characteristics make a clear point that the CE is not just one option to consider in the quest to meet climate targets, but it is the framework that will largely contribute to introducing the needed long-term solutions.

For better understanding of the concept of the CE (Kirchherr et al., 2017) the researchers offer „R“-frames beyond the basic „4R“ frame (reduce, reuse, repair, recycle), where the hues are most distinguishable (fig. 1).

Circular economy	Smarter use and production	**R-0 Refuse**	This could be achieved by making the product redundant by making its function redundant, or by offering the same function with a fundamentally different product
		R-1 Rethink	This could be achieved by making the use of the product more intensive
		R-2 Reduce	This could be achieved by increasing the efficiency of production or the use of the product by using less natural resources and materials
	Extending the lifespan of products and their parts	**R-3 Reuse**	This could be achieved by re-using a discarded product that is still in good condition and performs its original function by another user
		R-4 Repair	This could be achieved by repairing and maintaining a defective product so that it can be used according to its original function
Linear economy		**R-5 Refurbish**	This could be achieved by restoring and updating an outdated product
		R-6 Remanufacture	This could be achieved by using parts of discarded products for a new product with the same function
		R-7 Repurpose	This could be achieved by using a discarded product or its parts for a new product with a different function
	Useful application of materials	**R-8 Recycle**	This could be achieved by processing materials to achieve the same or lower quality
		R-9 Recover	This could be achieved by burning the materials to recover energy

Figure 1. 9R frame

*Source: Adapted from Kirchherr et al., 2017

While the basic idea of the CE (P. Planing, 2015) emerged in the 1980s, there are still no clearly defined business models aimed at the transition to a CE at microeconomic level. Establishing such a model requires a fundamental change in consumer behavior. The starting point in most of the research is the more efficient and extended use of existing products, recovery of the product or its components and materials, and energy recovery in order to prevent the landfilling of waste products. Also some models include using of components that last longer, paying for performance instead of a product, upgrading, reusing with or without treatment of the product, renting, leasing, paying for access instead for a product, conservation, recycling, maintenance, systems for products offered as services and sale after the end of the useful life of a product.

According to OECD (2019) the 5 key business models that could facilitate a transition towards a more CE are circular supply, resource recovery, product life extension, sharing and product service system. In the Circular supply model traditional materials should be replaced with renewable, bio-based or recovered ones while in the resource recovery model secondary raw materials from waste should be produced. Both the models should close the material loops. In product life extension models material loops are slowed by measures for extending the life of products. The sharing models and the product services systems models are narrowing the resource flow, respectively by increasing the utilization of existing products and assets and by providing services rather than products while the product ownership remains with the supplier.

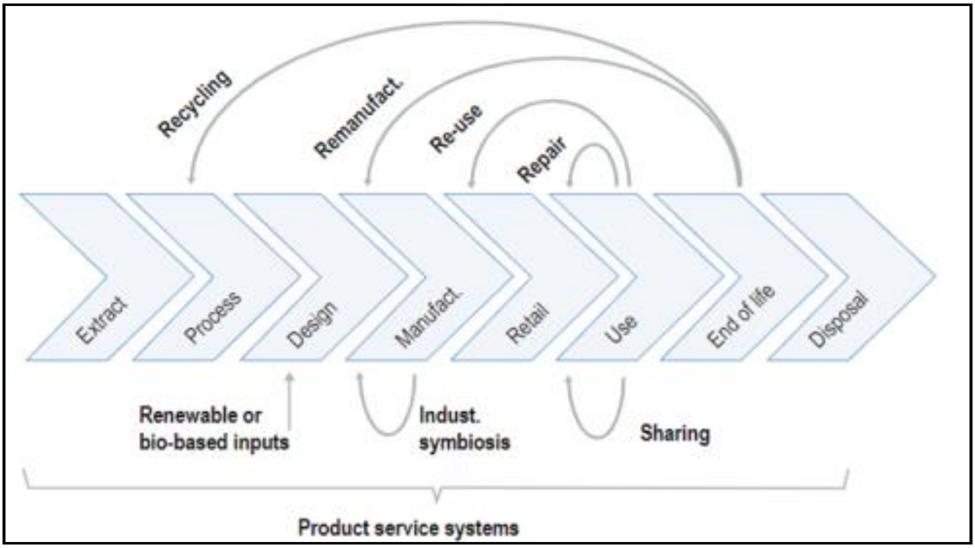

Figure 2**:** Impact of circular business models on different parts of the value chain of the linear economy

*Source: OECD, 2019, Business Models for the Circular Economy

In practice the distinction between these types of business models is not that clear as it is in theory but the main idea is the adoption of production and consumption methods that could contribute to lowering the pressure on the environment and have a great impact on the value chain of the linear "take–make–dispose" model (Fig.2).

Aguilar-Hernandez et. al. (2021) evaluate the potential impact on gross domestic product (GDP), employment, and CO_2 emissions by a review of more than 300 CE scenarios for the 2020-2050 time frame and reach to the conclusion that both in 2030 and in 2050 the implementation of ambitious CE scenarios could lead to an increase in GDP and employment levels, while substantially reducing CO_2 emissions. By implementing moderate CE scenarios the impacts on GDP and employment levels are negligible, while the CO_2 emissions would still decrease but with lower levels.

According to Arranz et. al. (2023) different consumption policies have a positive impact on the development of CE business models. Regulations on consumption modify consumers' behaviour and demand for products that comply with the CE while information raises the environmental consciousness and could be a driver for demand for circular products. At the same time, the impact of consumption policies is diminishing with the intensity of the policies so their continuous strengthening might lead to a decrease in the demand of circular products and the adoption of CE business models.

A research of Ellen MacArthur Foundation (2021) points out that 45% of the CO_2 emissions are related to production and by applying the CE models in five key areas - cement, aluminium, steel, plastics, and food - almost half of them or 9.3 billion tonnes of CO_2 in 2050 can be eliminated. Key in achieving this opportunity are business models that keep assets, products, and components in use, and make productive and efficient use of resources.

A recent study by Neves et. al. (2022) analyses the drivers and barriers in the transition from a linear to a CE by evaluating the role of economic, social, and environmental factors and confirms the importance for the transition to a CE. It is estimated that education and young-age are a precondition for increasing the use of circular materials, as well as the environmental awareness and environmental regulation, while old-age and income increases are a barrier. So for an effective transition to a CE, it is important for policies to target older and less educated people. Some other barriers for shifting to a circular model of production and consumption (P. Planing, 2015) are the customers` irrationality, the misaligned profit-share along the supply chain, the conflict of interests within companies and the geographic dispersion. The main reasons are that customers only evaluate the purchase price and prefer ownership, the imperfect product design result of the possibility that the profits from a better design would only occur at the end-of-use phase, the need of additional investments for changing the existing design and the low potential impact of national initiatives.

CONCLUSION

The CE is a response to the inefficient management of resources in the traditional linear model and it has the potential to increase the resilience to the effects of climate change by implementing its main principles of eliminating waste and pollution, guaranteeing circulation of products and materials and regenerating nature. Implementing an ambitious CE scenario leads to an increase in GDP and employment levels and reducing CO_2 emissions while in a moderate CE scenario the impacts on GDP and employment levels are minor, but the CO_2 emissions would still decrease. Some of the key business models that could contribute to a transition towards CE are related to ensuring circular supply, resource recovery, extension of the life of products, as well as sharing, offering a service instead of a product. In a circular system both the producers and the users have benefits as such a system could provide cost effective solutions on each level of the value chain. But while the basic idea of the CE emerged about 40 years ago, there are still no clearly defined business models to ensure the transition as they require a fundamental change in consumer behavior. Some barriers for shifting to a CE are the customers` irrationality, the misaligned profit-share along the supply chain, the conflict of interests within companies and the geographic dispersion, as well as old-age and income increases. On the other hand, the higher level of education, environmental awareness, environmental regulation and young-age are a precondition for increasing the use of circular materials. Along with the importance of developing appropriate business models to support the transition towards a CE, additional measures will be needed to help the transition to zero emissions and achieving climate neutrality.

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YIELD AND PROTEIN CONTENT OF GRAINLEGUMES INTERCROPPING WITH CEREALS IN TWO SPATIAL ARRANGEMENTS

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ABSTRACT

Intercropping of two or more species in the same field was a common practice in the past, butisstill followed by many growers in our days as well. The purpose of this research was to study the effect of two different spatial arrangements on yield after intercropping grain legumes with cereals. The experiment was established at the farm of the School of Agricultural Sciences, in Florina (Greece). For this to be done, two varieties of lentils (Elpida and Thessalia) were used as grain legumes, and bread wheat (cultivar Yekora) and oat (cultivar Kassandra) were used as cereals, which were planted individually as well as intercropped in two sowing systems (1:1 alternating rows and mixed rows in a 50:50 sowing ratio). In each experimental plot, six rows five meters long were sown, while the four inner were harvested. During the growing season, the following morphological traits were measured: the blooming, as well as the grain yield, and the protein content of the seeds. The protein content was determined by using the NIR (Near - infared - spectroscopy spectrometer). Differences were found between treatments regarding yield and protein content. The bread wheat variety Yekora and the oat variety Kassandra yielded better intercropped with the lentil variety Thessalia and Elpida respectively. Lentil variety Thessalia was not affected by the intercropping with Yekora in alternating rows, while it was damaged intercropped with Yekora in mixed sowing. The lentil cultivar Elpida was not affected by the intercropping with Yekora and Kassandra in either of the sowing systems. Regarding the protein content, the bread wheat variety Yekora was not affected by the intercropping with either of the lentil varieties in either of thespatial arrangements. The oat variety Kassandra wasfavored by intercropping with the lentil variety Elpida (mixed rows). However, Elpida was damaged intercropped with oat variety Kassandra (mixed rows).

Key words: *intercropping, grain yield, spatial arrangement.*

INTRODUCTION

Winter cereals are crops of great importance due to their ability to exploit poor, barren and mountainous lands, where no other crop would be economically viable (Papakosta, 2008).On the other hand, lentil (*Lens culinaris*) intended for human consumption, is a very important crop as well, because it is a rich source of proteins, minerals and vitamins for humans and animals' nutrition.Lentil has the ability to fix atmospheric nitrogen, so that its cultivation improves the concentration of nitrogen, carbon and organic matter in the soil, so that it is possible to integrate it into sustainable crop production systems (Sharker and Erskine, 2006; Vlachostergios et al. 2011).

Intercropping, a sustainable agricultural practice, is considered a promising solution to face the growing problems of food security and resource efficiency. Intercropping of cereals and legumes has numerous potential benefits, including enhanced resource utilization, increased biodiversity, effective competition with weeds, reduction of effects from enemies and diseases and improved overall productivity (Banik et al., 2006, Malezieux et al., 2009, Jensen 2020). However, there are some disadvantages of intercropping such as the competition for light, water and nutrients (Lithourgidis et al., 2008; Lithourgidis et al., 2011; Menbere et al., 2015). So, the selection of appropriate crop or varieties combination, the sowing system and the sowing ratio are crucial in order to minimize competition and to achieve the maximum yield (Sahoo et al.,2023). One critical aspect to be investigated is the influence of spatial arrangement on the intercropping outcome. Understanding how different patterns of crop arrangement, such as alternate rows, mixed rows, strip intercropping, or relay intercropping can affect grain yield and overall crop performance is an issue that needs to be investigated.

This research was undertaken to study the effect of two different spatial arrangements on yield and protein content after intercropping grain legumes, especially lentil, with cereals (bread wheat and oat) grown under low-input conditions and the possibility to apply these systems in modern farming.

MATERIALS AND METHODS

During the growing period 2020-2021, an experiment was installed at the farm of the University of Western Macedonia in Florina (Greece). The plant material used was cereals and grain legumes, which were cultivated in monocropping and intercropping. Particularly, cereals (greek bread wheat cultivarYekora, and oat variety Kassandra), were intercropped with two lentil varieties (Thessaly and Elpida). A completely randomized design with three replications was used. The 3 different species were cropped either as a monocropping or in a cereal-legume intercropping. So, a total of 30 experimental blocks were created. The above-mentioned cultivars of the three species (wheat, oat, and lentil) were intercropped in two sowing systems (1:1 alternate rows and mixed rows of the two species (wheat+lentil and oat+lentil) in a sowing ratio 50:50. Before sowing, basic fertilization was applied with the addition of diammonium phosphate (20-10-0), so that 80 kg and 40 kg ha^{-1} of nitrogen (N_2) and phosphorus pentoxide (P_2O_5) were added to the soil. In each experimental plot, 6 rows five meters long were sown, of which the four inner were harvested. The sowing distances between rows were 25 cm and a two-meter corridor was left between replications.The crop was kept free of weeds by hand hoeing when necessary.

The harvest of the four inner rows was carried out in July by combine harvester. During the growing season, the following morphological traits were measured: the blooming, as well as the grain yield, and the protein content of the seeds. The protein content was determined by using the NIR (Near - infared - spectroscopy spectrometer). Data were evaluated by analysis of variance (ANOVA) and the means were compared according to LSD test atp<0.05.

RESULTS AND DISCUSSION

Significant differences were recorded between the cultivars studied in seed protein content (significant differences at p=5%, Table 1). The % protein content, ranged from 5.34% for Kassandra intercropped with Elpida (alternative rows) to 31.1% for Thessalia (monocrop) (Table 1). Thessalia intercropped with Yekora (both spatial arrangements), Elpida intercropped with Yekora (mixed rows) and Kassandra (alternate rows) respectively, showed high protein content as well. Bread wheat variety Yekora showed the highest protein content both when it was grown in monocropping and in intercropping, in both sowing systems. On the contrary, oat variety Kassandra was favored by the intercropping with lentil variety Elpida (mixed rows).

Table 1.Seed protein content % in the 10 different treatments

Genotype	Cereals	Legumes
Yekora + Thessalia(alternative rows)	12.78*e	30.47ab
Yekora + Thessalia(mixture)	13.26e	30.17abc
Elpida		29.45bcd
Thessalia		31.1a
Yekora	13.08e	
Kassandra	5.4g	
Yekora +Elpida(alternative rows)	13.34e	28.96cd
Yekora +Elpida (mixture)	13.16e	29.95abc
Kassandra +Elpida (alternative rows)	5.34g	30.22ab
Kassandra +Elpida (mixture)	6.66f	28.6d

*Means followed by the same letter are not statistically significantly different for p<0.05

Statistically significant differences were observed between the genotypes studied in terms of yield (significant differences at p=5%, Table 2). The yield of cereals ranged from1064 kg /ha for oat variety Kassandra (monoculture) to 2504.0 kg/ha for Yekora (bread wheat), intercropped with lentil variety Thessaly in mixed rows. Regarding the lentil the yield ranged from 414kg/ha for Elpida intercropped with Yekora in mixed rows to 1465.0kg/ha for the mono-culture of Thessalia (lentil) (Table 2). Bread wheat variety Yekora was favored by intercropping with Thessaly (lentil) only in mixed rows. In all other combinations and sowing systems Yekora΄yield was not affected. Oat variety Kassandra was favored by intercropping with Elpida (lentil) in mixed rows. This means that the intercropping on the same row had a positive effect on grain yield of both Yekora and Kassandra. On the contrary lentil variety Thessalia was damaged by its intercropping with Yekora in mixed sowing, while it yielded well and was not affected by the intercropping in alternating rows. Elpida showed the highest yield in monocropping, while it was damaged by its intercropping with bread wheat (Yekora variety) and oat (Kassandra variety) in both sowing systems. However, this reduction was not statistically significant. Apparently, from the two intercropped species (bread wheat-lentil, oat-lentil) the lentil is the least competitive species.The above results suggest that intercropping did not affect the seed protein content of cereals and grain legumes except the oat variety Kassandra intercropped with Elpida in mixed rows, which was favored by this sowing system and lentil variety Elpida intercropped with Yekora in mixed rows that was damaged from this system.

Table 2.Grain yield of cereals and legumes grown in monocropping and intercropping in two different spatial arrangements

Genotype	**Grain Yield kg** ha^{-1} **(cereals)**	**Grain Yield kg** ha^{-1} **(lentil)**
Yekora + Thessalia(alternative rows)	1859.0bc	1443.0*cde
Yekora + Thessalia(mixture)	2504.0a	592.6gh
Elpida	-	801.9fgh
Thessalia	-	1465.0cde
Yekora	1287.0cdef	
Kassandra	1064.0efg	
Yekora +Elpida(alternative rows)	1504.0cde	516.0h
Yekora +Elpida (mixture)	1609.0cd	414.0h
Kassandra +Elpida (alternative rows)	1217.0def	597.0gh
Kassandra +Elpida (mixture)	2260.0ab	562.8gh

*Means followed by the same letter are not statistically significantly different for p<0.05

The superiority of the sowing in the same row compared to alternate ones, emerged from the data. This is in disagreement with Cheriere et al. (2020) who reported increased soybean production in alternate-row intercropping compared to mixed intercropping, between soybean and buckwheat, lentil, sorghum and sunflower. On the other hand Gennatos and Lazaridou (2021) reported that the grain yield of barley increased intercropped with forage pea only in mixed rows, but common vetch intercropped with barley was favored in alternate- row intercropping (Gennatos and Lazaridou 2021). Additionally Addo-Quaye (2011) reported that spatial arrangement greatly affected soybean growth in a maize-soybean intercropping system. In this study the variety Yekora (bread wheat) and Kassandra (oat) were favored by intercropping with the lentil variety Thessalia and Elpida respectively both in mixed rows. The increased yield of oat intercropped with lentil in mixed rows reported by Galitsas and Lazaridou (2020) as well. Lentil variety Thessalia was not affected by intercropping with Yekora in alternating rows, while it was damaged when intercropped with Yekora in mixed rows. Lentil variety Elpida was not affected by its intercropping with both Yekora and Kassandra in either of the seeding systems. In most cases, a higher yield was observed in the intercropping of the two species in mixed sowing rows compared to alternate rows. The above results suggest that there was a positive effect of intercropping concerning the cereals grain yield.

CONCLUSION

It was concluded that concerning the % protein content of the seeds, the lentil variety Thessalia contains the highest percentage of protein in all treatments.Intercropping did not affect the seed protein content of grain legumes except the oat variety Kassandra intercropped with Elpida in mixed rows, that was favored by this sowing system and lentil variety Elpida intercropped with Yekora in mixed rows that was damaged by this system.

Concerning the grain yield, the variety Yekora (bread wheat) and Kassandra (oat) were favored by intercropping with the lentil variety Thessalia and Elpida respectively both in mixed rows. Lentil variety Thessalia was not affected by intercropping with Yekora in alternating rows, while it was damaged when intercropped with Yekora in mixed rows. In most cases, a higher yield was observed in the intercropping of the two species in mixed sowing rows compared to alternate rows.There was a positive effect of intercropping concerning the cereals grain yield and it seemed that the cereals involved in the intercropping system had a better behavior when they were sowed as a mixture in the rows.

A first estimate of these results leads to the conclusion that sowing in the same row has a positive effect in the protein content seed production. However further research, including several seeding ratio and different cultivars, is needed to confirm the results of the present study.

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THE LANDSCAPE CONTEXT AFFECTS THE ECOLOGICAL VALUE OF AN ORGANIC FARM IN AN ITALIAN INNER AREA

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ABSTRACT

The general aim is to assess the landscape diversity and the ecological value of a multifunctional and diversified farm in an Italian inner area. The study was carried out in the context of the national DEMETRA research project, aimed at developing and implementing integrated and multifunctional agricultural production systems with a high degree of diversification and sustainability. For landscape context analysis, detailed land cover maps were produced and the composition and spatial configuration of the agricultural and natural landscape were quantified and evaluated. Moreover, using the i-Tree canopy software for rural areas, three ecosystem services were estimated. The results revealed a great diversity of land cover and use types, a small to medium size of the cultivated plots, and frequent contacts of cultivated areas with forest and semi-natural areas. This rural landscape supported the provision of numerous ecosystem services that resulted in a positive buffer effect for the quality of air, water and soil, ensuring great annual carbon sequestration, atmospheric pollution reduction, and consistently avoided runoff. Results highlighted the high ecological value of organic and multifunctional farms in inner areas and their contribution to face the environmental and food production challenges driven by global change.

Keywords: *landscape ecology, organic farming, landscape diversity, land use, ecosystem services.*

INTRODUCTION

Organic farming, characterized by a reduced environmental impact, has positive effects on soil quality, nutrient recycling, water resource management, biodiversity and other ecosystem services (Ciccarese & Silli, 2015; Abbott & Manning, 2015; Maitra *et al.*, 2020). A healthy agro-silvo-pastoral landscape can also absorb air pollutants, purify water, recharge aquifers, regulate the hydrological cycle, and provide recreational spaces and opportunities for psychological well-being (Scolozzi *et al.*, 2012). Multifunctional organic farms can therefore offer not only qualitatively and quantitatively better, resilient, and sustainable agricultural productions assuring many benefits to the community. In this context the scientific community has a key role on supporting farmers wishing to introduce agro-ecological innovations in both: implementing new approaches and on quantifying the related ecosystem services aiding their inclusion in the local and global green-market.

European inner areas host several ecological and multifunctional farms that maintain traditional landscapes and sustainable agriculture (Agnoletti *et al.*, 2019; Sivini & Vitale, 2023), and in Italy a good example of such landscapes are in the Abruzzo region (de Rooij, 2005; Grandi & Triantafyllidis, 2010). Abruzzo is an Italian region with high levels of biodiversity and is characterized by considerable ecological and environmental values (Bagnaia *et al.*, 2011). The high biodiversity value of the region is underlined by the large proportion of protected areas (37.2% of the region surface; both terrestrial and marine), the wide 58 Natura 2000 Sites and three National Parks (MASE, 2022a).

Abruzzo landscapes are characterized by wooded and semi-natural areas, in which deciduous forests, natural pastures and grasslands prevail (Pirone & Frattaroli, 2011). The utilized agricultural area (UAA) represents the 3.3% of the national agricultural lands (415,000 ha; Istat, 2022), and it includes the 2.4% of the organic farming in Italy (50,696 ha; CREA, 2021).

The study aims to give evidence of the positive effects of diversified ecological farms on ensuring highly functional (Goded *et al.*, 2019; Rotchés-Ribalta *et al.*, 2023), and heterogeneous landscapes (Brandt, 2003; Fahrig *et al.*, 2011; Stein-Bachinger *et al.*, 2022; Mannaf *et al.*, 2022) in the Abruzzo inner area (Central Italy). We specifically analyzed the landscape context of the VerdeBios farm present in an inner area where landscape is currently threatened by homogenization, mainly due to socio-economic dynamics linked to land abandonment of inland areas of the Apennines (Keenleyside *et al.*, 2010; Amodio, 2022), and whose presence over time may aid to contrast these threats. This article reports the results of an analysis on the landscape context diversity and the relative ecological value of the VerdeBios farm as well as the quantification of some ecosystem services. The target farm is characterized by a significant multifunctionality (agricultural production, zootechnics, pork processing, marketing in its own store, management of land for civic use, sale to solidarity purchasing groups).

MATERIAL AND METHODS

This study was born within the DEMETRA project (Ideation and validation of multifunctional and diversified production systems based on the integration between plant and animal production in the marginal areas of central-southern Italy), carried out by the BioCult Center of the University of Molise and funded by the MIPAAF competitive call referred to in DM 27/09/2018 n. 67374 (2020-2023).

It involves 5 farms that practice organic farming located in Abruzzo and Molise: Bio fattoria Licineto, Celenza sul Trigno (CH); Mancini Michelina Farm, San Salvo (CH); Opera Società Agricola Biodinamica di Vaira, Petacciato (CB); Terre del Seminario, Larino (CB); "VerdeBios", Celenza sul Trigno (CH).

The work focuses on the VerdeBios farm, that is located in Celenza sul Trigno village in the province of Chieti, Abruzzo, at about 646 m a.s.l., in an environment naturally favorable to organic crops, being characterized by a meso-mediterranean climate and a landscape with widespread high naturalness (Fig. 1). It has an extension of 42.45 ha and is composed of 85 cadastral parcels. Most of the farm plots are occupied by natural pastures and meadows, olive groves and arable land and deciduous forests, and sheep and pigs are raised there. The wooded areas consist mainly of *Quercus pubescens* with the presence of *Quercus cerris* and other tree species such as *Acer campestre*, *Fraxinus ornus*, *Sorbus domestica*, *Juglans regia* and *Ulmus minor*. In the undergrowth there are *Juniperus oxycedrus*, *Spartium junceum*, *Rubus ulmifolius*, *Cornus sanguinea*, *Euonymus europaeus*, and *Prunus spinosa*. In the warmer and steeper slopes Mediterranean scrub and ilex groves dominate.

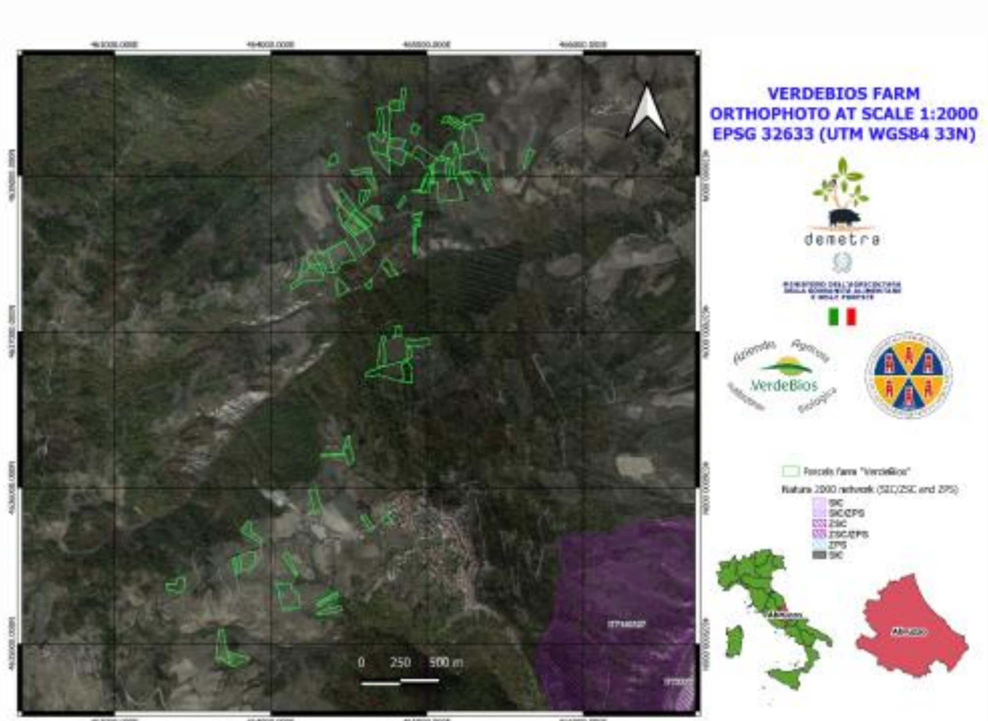

Fig. 1. Study area.

In order to investigate the landscape characteristics of the area, a map of the land use and cover of the VerdeBios farm parcels and the surrounding landscape was realized, considering a buffer zone of about 100 meters away from the farmland.

The cartography was carried out following the hierarchical scheme of the Corine Land Cover project at the third level of detail (ISPRA, 2018). Land use and land cover polygons were delineated and classified in a GIS environment (QGIS version 3.16) following a manual photointerpretation procedure of Google Earth digital orthophotos (year 2017) at a scale of 1:2,000 and assuming a minimum mapping unit (MMU) of 1,000 m^2 (< 0.1 ha). As ancillary cartographic information, the Regional Technical Map of the Abruzzo Region at a scale of 1:10,000 (Abruzzo Region, 2022), cadastral maps provided by the Italian Revenue Agency as a WMS service, and the Natura 2000 Network map (MASE, 2022b) were used. Validation of photo-interpretation was carried out through field checks.

Subsequently, to quantify and evaluate the composition and spatial configuration of the agricultural and natural landscape of the farm and the surrounding areas, ecological landscape indicators and diversity indices were applied (Kymberly, 2019; Huamaní Cahuas *et al.*, 2023).

Analysis was performed at the class and landscape level using Fragstats software (McGarigal *et al.*, 2012). The following metrics were calculated (Ferrari & Pezzi, 2013):

- Area of each land cover class in hectares (CA);
- Percentage of landscape (PLAND) of each cover class;
- Edge Density (ED) equals all edges in the landscape in relation to the landscape area and is an indicator of mosaic heterogeneity;
- Number of patches (NP) is the number of polygons of a given land cover or class;
- Shannon index and richness of patches that express landscape diversity in terms of richness of land cover classes and relative abundance (diversity index).

While, the percentage of land use and cover of the farm's contiguous areas (within 100 m of the farmland) was calculated using the following formula:

$$Pi = \frac{\sum_{j=1}^{n} a_{ij}}{A} * 100$$

Where: Pi is the proportion of the landscape occupied by category i; aij is the area in m^2 of category ij; and A is the total area of the landscape.

For the assessment of ecosystem services we adopted i-Tree software (USDA, 2021). The tools available in i-Tree assist technicians, private companies, and government organizations by quantifying the ecosystem services provided by rural and urban landscapes (Olivatto & Barduchi Barbin, 2017; Nowak *et al.*, 2018). The procedure taken as a guide was: Area delimitation -> Surface covers definition -> Surveying -> Report production (Olivatto & Barduchi Barbin, 2017). The analysis was performed through the i-Tree Canopy tool for rural areas, which consisted of uploading a shapefile of the VerdeBios plots on the online platform, then proceeded to perform a random sampling, placing 750 survey points (Olivatto, 2019; Selim *et al.*, 2023), detailing for each point to which surface cover corresponded (i.e., trees or not trees). We focused on climate regulation, air quality regulation, runoff mitigation/local temperature regulation.

RESULTS AND DISCUSSION

A total of 22 land cover classes were identified according to the Corine Land Cover legend at the third level of detail, identified by photo interpretation and field checks. Of these, 9 types fall in agricultural areas, 6 in artificial surfaces, 5 in forest and semi-natural areas and 2 in water bodies (Fig. 2).

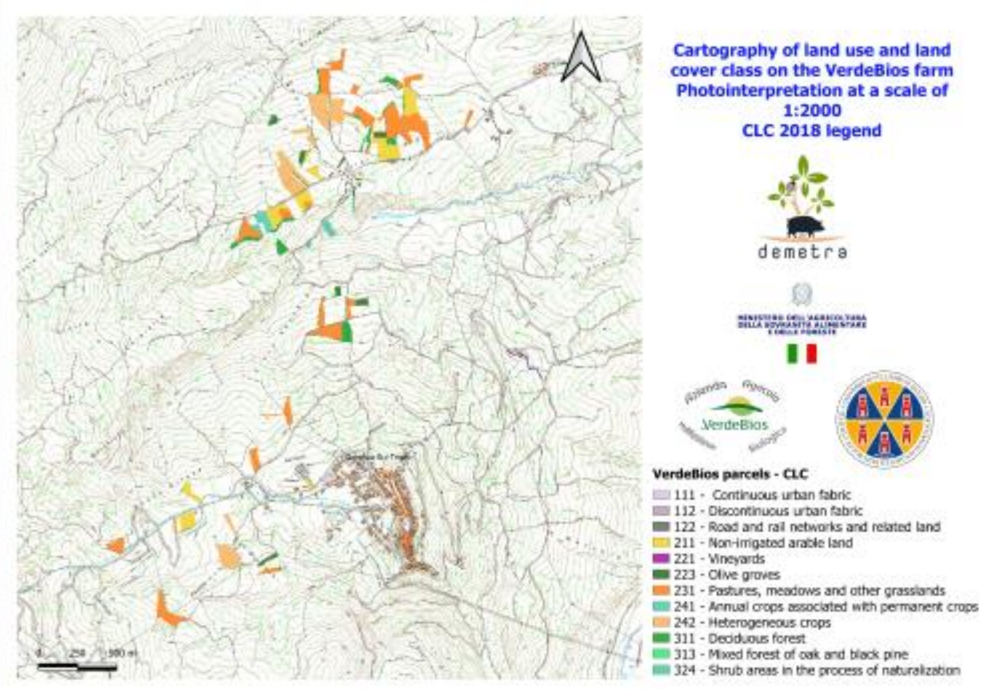

Fig. 2. Land cover map of the "VerdeBios" farm at the third level of Corine Land Cover legend.

There is a clear predominance of "Pastures, meadows and other grasslands" (class 231) covering 17.20 ha and representing 40.56% of the total study area, followed by "Heterogeneous crops" (class 242) with 9.05 ha (21.34%), "Non-irrigated arable land" (class 211) with 7.40 ha (17.46%) and "Oak and Cerro forest" (class 311) with 3.99 ha (9.40%). The Edge Density indicates that class 231 has a ratio of 98.46 m/ha, followed by class 311 with 62.61 m/ha, class 211 with 58.84 m/ha and class 223 with 39.26 m/ha. Regarding the average polygon size, the highest average value is class 242 with 0.82 ha, followed by class 241 with 0.51 ha, class 231 with 0.37 ha and class 211 with 0.35 ha.

A map considering a buffer area of 100 meters away from the cadastral parcels of the farm was made to know the composition of the land cover types in contact with the VerdeBios farm's estate (Fig. 3). The target farm is surrounded by almost 70% of other agricultural areas, but it is also in contact with forest and semi-natural areas (29%) and a very small number of artificial areas (4%).

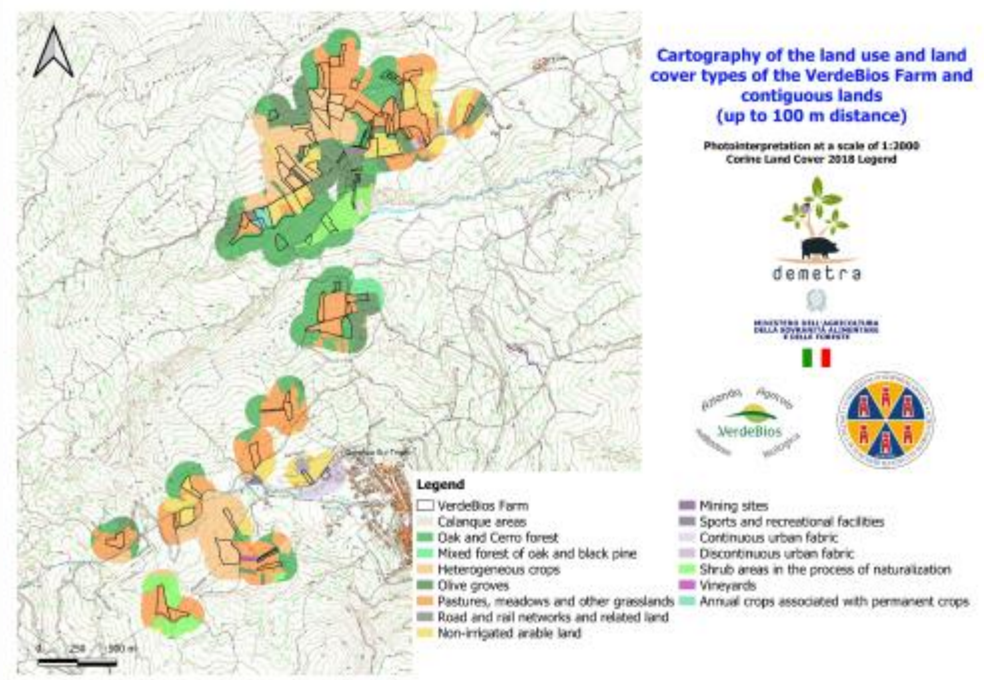

Fig. 3. Land cover classes (Third level of Corine Land Cover legend) occuring in the "VerdeBios" farm and contiguous areas (buffer of 100 meters).

As reported in recent scientific publications (Hass *et al.*, 2018; ISPRA, 2020; Samways *et al.*, 2020; Sirami & Midler, 2021; Tscharntke *et al.*, 2021; Barão *et al.*, 2022), the heterogeneity of the agricultural landscape, the small to medium size of cultivated areas, the diversity of land cover and use, and the proximity to natural and semi-natural environments increases the abundance of pollinators and maintains a high ecosystem biodiversity.

In general, an agro-forest landscape with widespread naturalness, such as the landscape context of the VerdeBios farm, supports the provision of numerous ecosystem services, such as the reduction of insect pests and insect vectors of pathogens, natural biological control, water and soil quality, and has greater resilience to the effects of climate change (Martin *et al.*, 2019).

In addition, the high spatial heterogeneity of agroecosystems as those observed in the analyzed area may increase the opportunities for multiple species to spatially segregate and locally coexist in equilibrium, thereby contributing to the maintenance of high levels of biodiversity and thus overall ecosystem functioning (Rosenfield *et al.*, 2022).

In contrast, a farm practicing conventional, intensive agriculture with non-diversified production is characterized by a homogeneous and therefore poorly functional landscape (Ekroos e*t al.*, 2010; Karp *et al.*, 2012; Carrié *et al.*, 2021; Sánchez *et al.*, 2022). Moreover, intensive farms, in order to improve their cultivation practices and facilitate mechanization, eliminate certain landscape

elements that provide indispensable ecosystem services (Emmerson *et al.*, 2016; Ribeiro *et al.*, 2019). The farm reality studied is strongly alternative to the process being underway of intensification of agriculture and homogenization of the landscape for the maximization of individual productive ecosystem services (production of food, fodder and biofuels; Maitra *et al.*, 2020).
These processes take place at the expense of other important services such as the provision of clean water, the maintenance of biodiversity and the loss of local knowledge and the identity value of places (Ferrari *et al.*, 2019). The high quality of the natural and rural landscapes is also associated with a tourist attractiveness that is lost in landscapes where agriculture becomes intensive (Mastronardi *et al.*, 2017). Several European studies show that multifunctional natural and rural landscapes are perceived as hotspots of ecosystem services (Di Cristofaro *et al.*, 2020) and provide multiple benefits for the well-being of different categories of stakeholders both locally and in neighboring cities (Garcia-Martin *et al.*, 2017; Fagerholm *et al.*, 2019).
The quantification of these services is useful for assessing environmental sustainability, promoting the farm itself, and making known its contribution to combating global changes (Lynch *et al.*, 2021). In that frame, the biophysical and economic valuation of some important ecosystem services provided by the landscape of the VerdeBios farm were calculated (Table 1).

Table 1. Biophysical and economic valuation of some important ecosystem services provided by the trees present on the VerdeBios farmland.

Ecosystem services	Indicators	Biophysical evaluation	Economic evaluation
Climate regulation	CO_2 Sequestration	112.28 ton/year	5,250 €/year
	Carbon storage	604.97 ton/year	103,706 €/year
Air quality regulation	Removal of air pollutants (CO, NO_2, O_3, SO_2, $PM_{2,5}$, PM_{10})	759.88 kg/year	43 €/year
Mitigation of runoff	Runoff avoided	315,16 liters/y	N/A

These services have relevance in the mitigation and adaptation to climate change and the reduction of surface runoff (Bengtsson *et al.*, 2019; Morizet-Davis *et al.*, 2023).

Trees contribute to climate change mitigation, because of their natural metabolic processes, they sequester atmospheric carbon dioxide by storing it during growth (Lorenz & Lal, 2010; Mistry *et al.*, 2019). The annual gross carbon sequestration (amount of carbon absorbed by the tree stand in a year) by trees on the VerdeBios farm is 30.62 tC/year, which is equivalent to 5,250.00 €/year. Carbon storage refers to the net amount of carbon stored in trees; that is, it is the total carbon that the plant has been integrating throughout its life (Di Cosmo *et al*., 2022). The trees of the VerdeBios farm store almost 604.97 t C/year.

Trees in rural landscape can help mitigate the impacts of climate change by regulating carbon sequestration and even reducing climate extremes (Vacek *et al.*, 2023; Ottaviano & Marchetti, 2023). Diversification of mixed production systems is of increasing interest as an adaptive approach to climate change, thus buffering risks to food production systems through increased livelihood resilience, food security and multiple ecosystem services (Baker *et al.*, 2023). For example, in agroforestry, trees can improve growing conditions for annual food crops by creating microclimatic effects (Morizet-Davis *et al.*, 2023). Moreover, trees help improve air quality by reducing air temperature and directly removing air pollutants from the air (Nowak, 2019). It is estimated that trees removed 759,88 kg/year of air pollutants (CO, NO_2, O_3, SO_2, $PM_{2,5}$, PM_{10}). Air in rural areas is of better quality than in many urban areas (Cvijanović *et al.*, 2017), and having a heterogeneous agricultural landscape can vitally help ecosystem processes in terms of air quality (Field & Parrott, 2017; Quandt *et al.*, 2023). The trees occurring in the VerdeBios farm contribute to the removal of pollutants such as carbon monoxide (CO), nitrogen oxide (NO_2), ozone (O_3), sulfate (SO_2) and particulate matter $PM_{2,5}$ and PM_{10}. The results show that trees contribute the most to the removal of O_3 (473,86 kg/y) from the atmosphere, secondly PM_{10} (187,99 kg/y) and thirdly NO_2 (53,16 kg/y). Air pollution is one of the main causes of respiratory diseases, so the elimination of polluting particles influences people's health (Manisalidis *et al.*, 2020). Moreover, elevated O_3 concentrations, coupled with climate change, could have negative effects on tree physiology (Takahashi *et al.*, 2020). Trees contribute to air purification, as a consequence of their functioning by helping to reduce the temperature of the surrounding air through transpiration; they facilitate the deposition of suspended pollutants on the plant surface (Nowak *et al.*, 2014). Trees can store atmospheric pollutants in wood, in annual growth rings (Alterio *et al.*, 2020). The economic value associated with the removal of PM_{10} is significantly high due to its direct relationship with lung disease affectations.

Trees and shrubs are beneficial by reducing surface runoff as they intercept precipitation. Surface runoff is the hydrological process at the origin of phenomena such as soil erosion, river flooding and mudflows that can generate significant damage. Surface runoff is that amount of rainwater that, during and after a precipitation event, is not intercepted by vegetation (trees and shrubs) and reaches the ground (Lagadec *et al.*, 2016; Guo *et al.*, 2019).

The results obtained show that the trees on the VerdeBios farm help reduce runoff by up to 315,16 liters/year.

CONCLUSIONS

Multifunctionality and ecosystem services have gained increasing interest in policy, as evidenced by key documents related to the Common Agricultural Policy 2023-2026, the Green Deal and the Farm to Fork strategy, and Nature-Based Solutions.

The analysis of the composition and spatial heterogeneity of the landscape associated with the VerdeBios multifunctional organic farm showed a high diversity of land cover types, a small to medium size of cultivated parcels and frequent contact with forest and semi-natural areas and pastures, delivering high provision of ecosystem services.

Trees present on farms have positive impacts on the environment, such as climate regulation, air purification and runoff mitigation; however, there is still a notable gap in the existing literature.

In this context, agro-ecological policy promotes configurational heterogeneity in European agro-ecosystems to increase functional biodiversity and ecosystem services provision, and the multifunctional organic farms may contribute significantly to reach that EC targets.

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INSTRUCTIONS FOR AUTHORS

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Results and Discussion should be combined into a single section.

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The International System of Units (SI) should be used.

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The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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