**FEB - FRESENIUS ENVIRONMENTAL BULLETIN**
Founded jointly by F. Korte and F. Coulston
Production by PSP - Vimy Str. 1e, 85354 Freising, Germany in
cooperation with PRT-Parlar Research & Technology - Vimy Str 1e, 85354 Freising

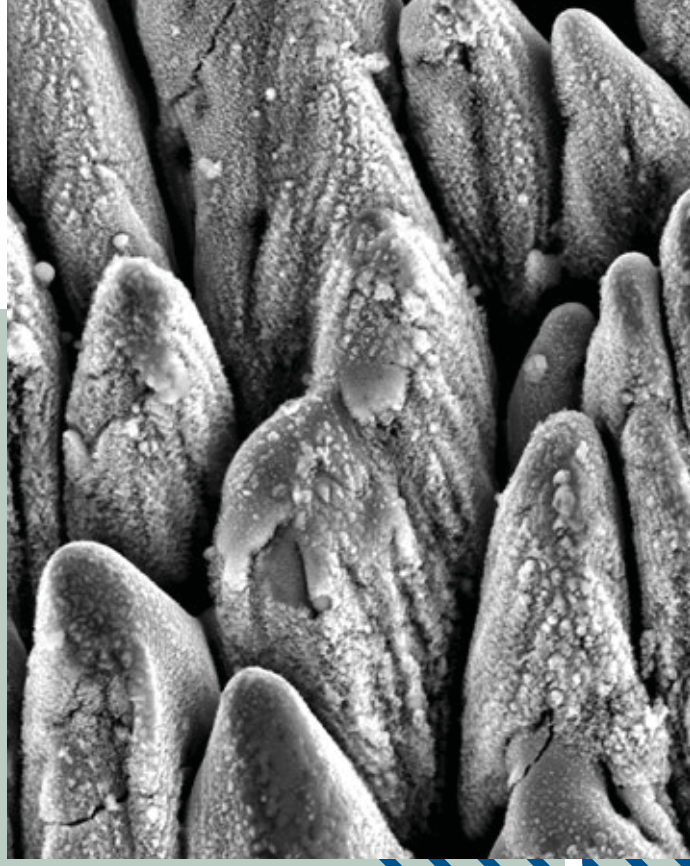

**Printed in Germany-ISSN 1018-4619**

## FEB-EDITORIAL BOARD

**Fresenius Environmental Bulletin is abstracted/indexed in:**
**Biology & Environmental Sciences, C.A.B. International, Cambridge Scientific Abstracts, Chemical Abstracts, Current Awareness, Current Contents/ Agriculture, IBIDS database, Information Ventures, Research Alert, SciSearch, Selected Water Resources Abstracts, EBSCO**

# CONTENTS

EUROPEAN SYMPOSIUM ON
# Surface Science



# 4th Edition
# April, 17-19, 2024
# Athens / Greece

**With focus on**
Science and Technology of Material Surfaces

**Organized by**
The European Association of Material Surfaces Science and Technology

*European Association of Material Surfaces Science and Technology*

# Table Of Contents

# About the Conference

The conference aims to act as an interdisciplinary forum for research scientists and interested people and experts from all scientific disciplines of politics and economics to examine and discuss the current state of the art of material surfaces, including natural surfaces.

Previous conferences were held in Rome - Italy (2014), Capri - Italy (2016), and Nice - France (2020).

Key scientific/technological topics of the symposium

- **Smart Coatings and Surface Modification,**
- **Nanotechnology and Nanomaterials,**
- **Advanced Manufacturing/Processing and Characterizations.**
- **Natural surfaces**

as applied in:

- **People's health and well being**
- **Safe, smart, green transportation**
- **Energy production and management**
- **Smart cities: quality of the urban environment**
- **Impact on environment and climate**

# Conference Overview

The program of the conference will include keynote lectures, voluntary oral presentations and posters. Keynote lectures 30 minutes, voluntary oral presentations 20 minutes plus 5 minutes for questions. All presentations (oral and posters) should be performed in English language. Selected posters will be presented in short (5-minute) oral sessions in full audience.

## PAPERS PUBLICATION AND ABSTRACT SUBMISSION

For contributions, a one-page abstract should be submitted using the abstract form present at the end of this circular or visiting the website www.emasst.com no later **than December 20, 2023** to the following address: info@emasst.com with “Abstract for European Conference” as subject.

Authors will be notified on acceptance and on the form of presentation of their contribution by **January 15, 2024**.
All participants interested in the topics of the conference are invited to register until **January 25, 2024**. The book of abstracts will be distributed on electronic media to all participants upon registration at the Symposium.
Further information about the publication of short papers (4-5 pages) will be communicated later.

# Conference Details

## REGISTRATION

Symposium participants may register before or at the Symposium. Please fill the registration form present at the end of this circular or download at the website www.emasst.com

## REGISTRATION FEE

| | Early Registration (€)* | Late Registration(€)** |
|---|---|---|
| EMASST- Member | 150 | 200 |
| Non EMASST- Member | 200 | 250 |
| Student | 100 | 150 |
| Accompanying person | 75 | 100 |
| **EMASST membership 50€ (per year)** | | |

*Early registration (until December 20, 2023)
** Late registration (after December 20, 2023)

*The registration fee includes the final program, the book of abstracts in electronic format, access to the hospitality desk, coffee and refreshments during the symposium, the welcome cocktail, social dinner and certificate of participation. Accompanying guests will be entitled to the welcome cocktail and social dinner. Undergraduate and graduate students can register at a reduced fee if they provide a formal letter certifying their student identity from their academic institution.*

# Conference Roadmap

December 20, 2023 Abstract submission deadline
January 15, 2024 Notice to Authors on the acceptance and form of presentation
December 20, 2023 Deadline for payment of early registration fee

The **Amalia Hotel** in Athens will be the conference location

Hotel reservation can be done at the Amalia Hotel (near by the Central square Syntagma in Athens), on the first come-first served basis with a discount of 18% using the form below:
In order to proceed with reservation, guests should visit our website's booking engine via the following link : https://amaliahotelathens.reserve-online.net/

Then choose dates & add **BOOKING CODE : EMASST** in order to gain 18% discount from our official rates.

Alternatively, please book the hotel on your own.
Detailed information on transportation will be given in the 2nd Circular by the end of January 2024.

# Conference Team and Contacts

## Organizing Committee

Dr. Dimitrios KOTZIAS (Chair)
Dr. Rosanna MABILIA
Dr. Paolo CICCIOLI
Dr. Daniele M. TRUCCHI
Dr. Virginia NOVIELLO

## Steering Committee

Dr. Dimitrios KOTZIAS (Chair)
Dr. Rosanna MABILIA
Dr. Paolo CICCIOLI
Dr. Daniele M. TRUCCHI
Dr. Luca MAZZOLA
Prof. John BARTZIS
Dr. Vassilios BINAS
Dr. Nadja FELDE
Dr.Alessandro BELLUCCI
Prof.MariaPia PEDEFERRI
Prof. Georgios KIRIAKIDIS
Prof. Harun PARLAR
Prof Panos PAPAGIANAKOPOULOS
Prof. Georgios PILIDIS
Prof. Miltiadis KARAYANNIS
Dr. Ettore GUERRIERO
Dr. Benedetto PIZZO

## INFORMATION AND CONTACTS/LINKS

For further information please send an e-mail message to:
info@emasst.com

Links: www.emasst.com

-© by PSP Volume 32– No. 09/2023 pages 2936-2953 Fresenius Environmental Bulletin

# ATATÜRK'S HIGHER EDUCATION REFORM, FORESTRY EDUCATION AND ONE OF THE MILESTONES OF THIS EDUCATION; PROF. DR. ABDULGAFUR ACATAY AND HIS 120TH. BIRTHDAY

**Ilhami Kiziroglu***

Ostim Teknik Üniversitesi, 100.Yil Blv 55/F,06374-Ostim Osb/Yenimahalle-Ankara, Turkiye

## ABSTRACT

Discussed is the emergence of the university reform in 1933, which is one of the most important stages of the revolutions initiated by Atatürk. In this context, the foundation of Istanbul University and the curriculum at Ankara Higher Agricultural Institute, which will form the Ankara University to be opened later, has been established with German scientists contracted from Germany or persecuted by the pressure of the Nazi regime in Germany, committed to the university with a contract. The group of these German scientists have founded an association in Switzerland and as imigrants they aspire to university posts in various countries of the world. The process of assigning those who are committed to the university institutions in Istanbul and Ankara with a contract is emphasized. In this context, the contributions of Prof. Schwartz and Prof. Malche are mentioned and highlighted. In the light of all the developments, it has also been shown how the forward-looking and comprehensive vision ideas of the great leader of the Turks Gazi Mustafa Kemal Atatürk were true with regard to universityization. After providing information about the development of forestry education, which is one of the most important units of YZE, before and after the decision of the Faculty of Forestry to continue education at Istanbul University, the development of forestry education. Information was given about the faculty members of the first Turkish generation of the Faculty of Forestry who were educated abroad. An important authority in forest entomology, forest conservation, wildlife, hunting and botany of this first generation was Prof. Dr. Abdulgafur Acatay. An attempt has been made to give the important achievements and information about the accomplishments of him within the framework of his 120th birthday and his life story.

**KEYWORDS:**
Atatürk's university reform, German guest lecturers, forest education; Forest entomology and forest protection.

## INTRODUCTION

In this study, Atatürk's higher education reform; that is, the transition from the old university studies (Darülfunun) to the university process and specific information about the foreign visiting professors who have done very important and lasting work in this framework are briefly given [1-16 ]. In addition, brief information is provided on the development of Turkish forestry education and the foreign and local founding scientists who have been involved in this process [17-23]

Atatürk's university reform was a very important modern reform of Turkish higher education. It opened an excellent modern university education window. So, in this study, the process of this development; the development of Turkish forestry education in the initial phase, where both the foreign and local teachers who participated in this process and passed their graduate forestry studies and not least their doctoral studies with great success and returned to Turkey, and in the initial phase forestry education contributed a lot. One of them is Prof. Dr. Abdulgafur Acatay, who is one of the milestones of forest entomology and forest conservation science, has high reputation both in the national and international scientific arena. As his students will celebrate his 120th birthday in 2023, his forestry achievements have been reported in this study using his available research sources.

Atatürk emphasised universities, students and faculty members at every opportunity. He wanted the universities to be structured and developed according to the needs and requirements of the modern world. He always supported competition with foreign principles in higher education and the conduct of scientific studies and empowered the then Ministers of National Education to do what was necessary. At the time of the proclamation of the Republic, the number of schools and students at all levels was very small (for example, there were a total of 1241 students in 23 high schools throughout the country, rising to 82 high schools and 25 thousand students in 1938); there were only 6547 students in

64 vocational and technical schools. In 1923, the year of the establishment of the Turkish Republic, 9 higher education institutions were in operation, including Darülfunun. This number increased to 20 in 1938 and the number of students increased to 13 thousand [12,13]. According to Meydan [13], when people think of university reform in Turkey, they think of the closure of the Darülfunnun in 1933 and the opening of Istanbul University in its place; however, when Atatürk speaks of university reform, he means a renewal and mobilisation of education that encompasses the whole country from primary to higher education, and the establishment of modern universities and colleges in many parts of the country within this framework. In essence, Atatürk's so-called university reform is not only a university reform but also the final culmination of the cultural movement that Atatürk had started in 1925 and 1926[5,13]. With the idea that the concept of the university could only be possible through the education of young scholars, he paved the way for 42 students in the academic year 1927-28, 170 students in the academic year 1928-29 and 288 students in the academic year 1929-1930 to study abroad and return home after completing their doctorates after the establishment of the Republic[24]. With the return of these students to the country, the University Reform (UR) was initiated.

This torch of education lit by our great leader **Gazi Mustafa Kemal Atatürk** was expressed in the memoirs of Hasan Rıza Soyak as follows: -*" The days are approaching when the children who are in education or internships inside and outside the country will grow up and find employment one by one. In this regard, we can assume that the time is not far when the problems we have suffered due to the shortage of labour will subside"*[8,10]. Our great leader Gazi Mustafa Kemal Atatürk, as with most of his important breakthroughs, wanted Darülfünun, which opened in the Ottoman Empire on 12 January 1863, closed four times and reopened in 1911, to develop into a university so that the university would have a modern identity and produce science at a level that could compete with equivalent institutions abroad. When Atatürk could not see a modern breakthrough in Istanbul's Darülfünun, he decided to enrol in a two-year programme in 1931.

A "university reform" that would last for a long time. For Gazi Mustafa Kemal Atatürk realised that the most important problem in the process of founding, renewing, organising and restoring the Young Turkish Republic was the lack of educated people in higher education. He knew that the revolutions he wanted to realise would be sustainable in the light of science. He believed that the only way for Turkish society to advance in production, industrial development, economic and cultural development in accordance with the demands of the times, and for this to be internalised in all strata of society, was through the universities. There was a science-hungry youth, and he realised that their education would not work at the old universities. To remedy this situation as soon as possible, the establishment of a university with modern features had become the most important necessity. For this reason, Prof. Dr. Albert Malche was invited to Ankara. Prof. Malche prepared a 95-page report with 49 articles on 29 May 1932. In the "Report on Istanbul Darülfünun", education in Istanbul Darülfünun was assessed as medieval and it was stated that there was no space for research, speaking and thinking (8,24). This report highlighted the lack of libraries and the lack of cooperation between the existing libraries, the inadequacy of the collections, the low budget and many other problems [10]. The great Atatürk read this report, which was to form the basis for university reform and the establishment of a new university in Turkey, line by line and took notes; however, he found Malche's proposals on higher education insufficient and spoke of a more comprehensive "cultural planning" [12,13]. Atatürk's modern university project looks like this:''- *A real university dealing only with science, analysing Turkey's geology, natural and economic geography, climate, flowers and plants, anthropology of land and sea animals, history, industry and culture*''. In doing so, he emphasised another point: ''-*even foreign geniuses cannot save us; it will be we ourselves who save us. We can benefit from the views and thoughts of foreigners, but we must be very careful in doing so*". Within this framework, Dr. Reşit Galip, the young and dynamic Minister of National Education, was instructed to implement the university project as soon as possible and a commission was set up. Prof. Malche's report was adopted as the university reform programme. Darülfünun was closed and the legal foundations of Istanbul University (IU) and later Ankara University were established on 31 May 1933 with the approval of the Grand National Assembly of Turkey. After the relevant law was passed, the IU was established on 1 August 1933 and started its educational and training activities on 19 November 1933. In 1927-1930, 501 students who were sent abroad for education completed their studies or doctorates and were employed at the IU from 1932-1933. In the first academic year 1933-34. While the number of students who began their studies at the university was 3 417, this number tripled to 10 178 in 1943, almost 50 per cent of whom were female students [12].

The most important event in the framework of scientific relations during the Republican period was the visit of 14 German agricultural experts to Turkey in 1928 under the leadership of Prof. Oldenburg and the foundation of the Agricultural College in Ankara in 1930. This school, which adopted the same educational system as the agricultural faculties in Germany, was transformed into the Higher Agricultural Institute three years later, in the year of

the university reform. The lecturers working at the Higher Agricultural Institute (YZE) in Ankara were appointed under a bilateral agreement with the German government. During this period, education continued at the YZE in Ankara in the departments of forestry, agriculture and veterinary medicine [15,16,25]. The main question was how to overcome the lack of human resources to work in these institutions and to produce and teach science. At this point, Atatürk's foresight, his aspiration for high realisation and his ability to solve problems came into play: Atatürk, who was a keen observer of the European and world economy, had received news in the early 1930s, before the Second World War, that scientists of Jewish or non-Jewish origin who had been subjected to repressive persecution by the Nazi regime and opposed its rule had begun to leave their countries within a very short time.... Indeed, at some universities in Germany, the textbooks of Jewish, communist and anti-government academics were first burned and then their lives threatened. For example, many lecturers at Europe's most important university, Heidelberg, were forced to flee abroad. Prof. Dr. Philipp Schwartz left his country with his family and came to Zurich, where his father-in-law, the biologist Prof. Dr. Sinai Tschulok, lived. With the support of his friends, the neurologist Prof. Dr. Erich Katzenstein, Prof. Dr. Kurt Goldstein, some Jewish businessmen and his father-in-law, he founded the "Emergency Community of German Scientists and Artists Abroad". Scholars Abroad) [2-4,6,11, 15,25-28].

This information reached Ankara as if it was the kind of news Atatürk was looking for to solve the university problem but could not find. Prof. Schwartz's father-in-law, Prof. Dr. Sinai Chulok, was friends with Prof. Dr. Albert Malche, for whom Atatürk had prepared the report on higher education in Turkey [2,28]. Prof. Malche contacted Prof. Schwartz and guided German scientists to Turkey. Prof. Malche and Prof. Schwartz met at the Ankara train station and then attended a meeting with the delegation led by Dr. Reşit Galip, the Minister of National Education. The language of the meeting was French, because in those years French was the language of diplomacy and science in Turkey. At this meeting, the professors to be hired at universities in Turkey were selected and a) Their salaries, b) Personal rights, c) Learning the Turkish language within two years, ç) Writing books in Turkish and d) The condition that each scientist train young Turkish assistants in his field were discussed and decided. Prof. Schwartz was very pleased with the outcome, he sent a telegram to Zurich and expressed his joy with the words *"-Not three, but thirty scientists"* in his telegram. The best-known scientists who came included Ernst Reuter, Fritz Neumark, Ernst Eduard Hirsch, Hans Reichenbach, Leo Spitzer, Erich Auerbach, Ernst von Aster, Marchand, Wilhelm Röpke, Gerhard Kessler, Philipp Schwartz, Rudolf Nissen, Alexander Rüstow, Friedrich Dessauer, Richard von Mises, Rudolf Belling, Curt Kosswig, Bruno Taut and Paul Hindemit. Ernst Reuter, together with **A. Rustow, G. Kessler, C. Kosswig**, founded the *"German Freedom Association"* in Istanbul in 1943, which repeatedly denounced and condemned the attitude of the Nazi administration towards scientists [14]. These scholars made significant contributions to the development and scientific path of Turkish academic life. Some of them made significant contributions to the modernisation of Turkish academia through the students they educated. Some of them worked at Istanbul University and some in Ankara.

They took office in the institutions. The parties agreed that the professors should be in Turkey by 15 October 1933, which was set as the date for the start of their education [1-4,9,12, 27]. The staff of Istanbul University, which was established by Law No. 2252 (1933), is composed of three different sources:

**A) Those from the Darülfünun(old higher edicational school); B) Those who returned home from the young people sent to study in Europe; and C) Professors appointed from Europe.** In this modernisation movement that followed the university reform in higher education, valuable scholars who came to Turkey tired of the oppression of Nazi Germany made an important contribution. Prof. Hirsch and Prof. Neumark learned Turkish very quickly. As soon as it was possible, they started giving exams and then lectures in Turkish, and later became the first German scholars to write their books in Turkish [9,14,15] notes that not only German scientists of Jewish origin came in this context, but also their families, relatives, assistants, medical and technical staff. At least 500-600 scientists, including full and associate professors, assistants, lecturers and support staff, who had to leave Germany, formed a group of 1500 people together with their families. In 1933, one hundred and fifty people, including eighty-two professors, arrived within two months: between the 1930s and 1940s, they taught at the Istanbul and Ankara Higher Institutes of Agriculture (AYZE) and the later Ankara Universities (in addition to academics, translators, lecturers, teachers, specialists, workers and self-employed persons; a total of 481 people came to Turkey [9]. According to Widmann [1], 27 Turkish and 38 foreign full professors, 18 Turkish and 4 foreign professors, 93 Turkish associate professors, 99 Turkish assistants, 43 foreign assistants and a total of 237 Turkish and 85 foreign (German) teaching staff were employed at Istanbul University from 1933 onwards during this period [11]. Around 26.3 per cent (85) of the 323 people who took up employment at universities were of German origin. Due to the contribution of scientists and artists of German and Austrian origin, Turkey

became an important refuge for the **"intellectual elite"**) [6,14,16,26].

At the end of their three-year term, 70 of them emigrated to the USA or other countries. The "University Reform" of 1933, carried out under Atatürk's leadership, brought an understanding of the system to our higher education administration, defined the tasks of universities in a clear and contemporary way, and led to the introduction and establishment of international terms such as (university; rector; faculty; dean; professor; associate professor; assistant, etc.) in the higher education system. The modernisation of higher education in Turkey began with the "university reform" carried out by our great leader Gazi Mustafa Kemal Atatürk in 1933. This process was not easy for the German academics who came to Turkey. For although they began working abroad and drew their salaries from a second country, the control of the Nazi government continued uninterrupted. The report prepared by Dr. Herbert Scurla, which was produced by the German government to put pressure on the arriving German scientists to check whether they were loyal to the government or not, is a document demanding control over whether the German scientists who found refuge in Turkey served the Nazi ideology or not, and the persecution of the Jews. The fact that the Turkish administration at the time expressed very seriously and clearly to the German administration that this view cannot apply here, and confirmed and affirmed that the German scientists are under the umbrella of all kinds of protection of the Turkish government against the power of this person, is extremely important for the visiting scientists and their families. This situation shows, as Şen (15) states, that it should not be forgotten that the administration of the Turkish Republic made a unique effort after 1933 to protect the cultural heritage of free and world-loving Germany. In fact, this attitude was crucial, as it acted as a protective and supportive element for the German scholars of Jewish origin, known as Muhajir, most of whom resided in Istanbul, in the establishment of Istanbul University. Therefore, thanks to the protective patronage of the Turkish state, the National Socialists could not take any negative action against a German scientist working in Turkey during this period. Neumark (2) states that Dr. Scurla, who came to Turkey twice in 1937 and 1939 and worked as a political agent, *"-did not possess a brilliant intellect, but was an agitator"*. The Turks could not fail to see the real motives behind the transfer and dismissal proposals that Scurla made to them. I feel a deep sense of gratitude towards the Turkish government of the time and my Turkish colleagues who sided with us (2).

Atatürk's new university report should be seen as the most important practice in the history of Turkish higher education, a kind of recruitment of humanities scholars. During this period, a modern university organisation was established, the young generation of scholars was trained, world literature was opened up, and many new scientific developments and modern approaches were introduced, from health sciences to agriculture and forestry. The publication of scientific journals was started, publications on experimental research were given high priority, and very important tasks and missions such as enabling cooperation between public authorities and universities and meeting the "University Weeks" held in a different city every year were put into practice. In the Faculties of Medicine, Natural Sciences, Literature, Law, AYZE Forestry and Agriculture and Economics affiliated to Istanbul University, which was opened in place of Darülfünun, visiting scholars began to work with great dedication and wide-ranging powers and achieved significant successes. Thanks to the foreign scholars, the teaching programmes were adapted to the times and Istanbul University ranked second after Cambridge University in the 1937 ranking of world universities [29]. A large number of these foreign scholars were invited and sent to our country within the framework of the great foresight, mission and high vision of our great leader Gazi Mustafa Kemal Atatürk. Thus, a large number of professors working at the universities of Istanbul and Ankara have made a great contribution to the cultural development of the country. Neumark (2) noted that by the end of his term we had also educated a sufficient number of Turkish successors. In fact, the introduction of the Latin alphabet by Atatürk was in itself an important practice of cultural development and the most important revolution that enabled us to work here (2).

The Ottoman Empire brought two valuable forestry experts from France in 1856 to support the treasury's revenue sources, manage the forests, determine the applicable technologies and improve forestry education. As a result of the joint efforts of foreign and local experts, the first forestry school was opened in Istanbul in 1857, but it met with little interest because it taught in French; however, it was closed in 1862 and reopened by Tassy in 1866 [20,22,30,31]. The Directorate General of Forestry(OGM), where graduates of the school were to be employed, was founded in 1869, and in 1870 the Forestry Ordinance was published, establishing the scientific basis for the profession of forestry. In 1880-1893, this school was transformed into the Forestry and Mining School, then the two schools were merged and the Forestry and Mining School was opened, with the addition of some Turkish teachers to the teaching staff. Thus, the language of instruction was partly changed to Turkish and graduates were given the title of "forest engineer". The first two years were divided into joint departments, the next two years into forestry and mining departments. In place of this school, which was closed by a resolution in 1893, there was the

**TABLE 1**
**Evolutionary development process in higher forestry education after Unireform of Atatürk.**

| School Name | Place | Opening Year | Graduation Year | Duration | Semester |
|---|---|---|---|---|---|
| YZE Faculty of Forestry * | Ankara/Istanbul | 1934 | 1948 | 14 | 04. Apr |
| Istanbul University Faculty of Forestry | Istanbul | 1948 | 2018 | 70 | 8 |
| Istanbul University-Cerrahpaşa Faculty of Forestry** | Istanbul | 2018 | | | |
| Other Forestry Faculties (1971 in Black Sea TU; from 1992, Forestry Faculties were opened in Artvin, Bartın, Düzce, Isparta, Kahramanmaraş, Kastamonu, Çankırı, Bursa, İzmir and Karabük, the number of which has increased to 12)**. | | | | | |

*) Law No. 2291 of 10.06.1933 established the Higher Agricultural Institute, an agricultural university. The College of Forestry was integrated into the Higher Agricultural Institute as the Faculty of Forestry by Law No. 2524 of 18.06.1934.
**) The employment opportunities of the graduates should have been calculated and the number of quotas allocated to the newly opened faculties by dividing the number of available quotas (we would like to emphasise that the mistake made in some departments of the Faculties of Agriculture and Natural Sciences, such as Biology, should not be made in the Faculties of Forestry). If an increase in the number of forestry faculties would mean an increase in the number of forests, shouldn't there be many more forestry faculties, not 6 in Russia, 7 in Brazil, 8 in Canada, 4 in Germany, 2 in Sweden.... It would be useful to reduce this number in our country and calculate the number of students to be admitted at the regional level and the number of students to be admitted in 10- and 20-year periods and reduce them accordingly.

Halkalı Agricultural High School from 1893 to 1903 and the Halkalı Agricultural and Forestry High School from 1903 to 1910. After that, the forestry education given here was deemed insufficient and forestry education was taken up in the Forestry School (Mekteb-i Alisi) founded by **Hodja Ali Rıza Efendi** in 1910, which existed until 1934. The influence of Hodja Ali Rıza Efendi, who also took over the OGM in the same period, on the independence of the forestry school is important [22]. The duration of education at this school was first increased to 4 semesters and then to 6 semesters, following the example of German schools [21].

In the first phase of forestry education in Turkey, French influence lasted until the First World War. In the period that followed, the significant contribution of German and Austrian scholars and opportunities for collaboration came into play [15,18]. In this framework, especially during the period of the AYZE(Table 1), the information about the scientists, their countries and subjects that contributed to the continuation of forestry education both in Ankara and Istanbul at the Faculty of Forestry is presented in Table 2. Forestry education the period of 1933/34, the year when forestry-agriculture and veterinary education started within the framework of the Higher Agricultural School opened in Ankara, is significant. This is because many German scientists were involved in this higher education institution, which was headed by Prof. Dr. Gustav Oldenburg. The first rector of this institution was Prof. Dr. Friedrich Falke. Prof. Gleisberg, who took over as director, divided the institution into three technical schools (corresponding to the faculties of agriculture, forestry and veterinary medicine), which were primarily oriented towards practice.

The German scientists working here, with the exception of Prof. Gerngross and Prof. Richter, were sent to Turkey with the permission of the German government; in a sense they were all civil servants. In fact, many professors working in Turkish universities today are either students of these foreign scholars or students of their students [11]. Some of the German/Austrian scholars listed in Table 2 escaped the repressive Nazi regime in Germany and came to Turkey and made significant contributions to various professional education and arts organisations in Turkey [1,15,32]. These were important scholars who later formed the main pillar of forestry education and contributed to the careers of the Turkish scholars mentioned in Table 3, who also taught us, the previous and the next generation. The first two years of training in the Department of Forestry at YZE Forestry were completed in Ankara and the next two years in Istanbul; however, later the Faculty of Forestry was established at IU; however, later the IU Faculty of Forestry was established. In 1933-34, the first two years of forestry training were completed in Ankara and in Bahçeköy, Istanbul Table 3. The indispensable part of forestry practices and observations was the exploration of impenetrable forest ecosystems. Especially in the 1930s, it was very difficult to conduct observations in the forest without horses (Figure 1). It is even known that there were horses and pack mules in this system all the time.

In the absence of a Faculty of Forestry, the pioneers of Turkish forestry education started working as forest engineers after graduating from the Forestry Engineering Department or the six-semester Forestry College within Ankara Higher Agricultural School (AYZE). Thanks to the foresight of our great leader Gazi Mustafa Kemal Atatürk, those who had received scholarships to study abroad were sent to some European countries, especially Germany. They first completed an 8-semester course of study in forestry engineering and then completed their doctoral training and returned home with the title of Doctor of Forestry Engineering. Table 4 shows the information on the universities where these scientists, who were the milestones in the

establishment of the Faculty of Forestry, completed their undergraduate studies and then their doctoral studies. These scientists wanted the research carried out in the establishment phase of higher forestry education to be completed in a short time. In 1933, as part of the initiatives on the future of the AYZE, which was left out of the process of transforming it into a university, it was emphasised that it should be considered within the university, and in the 1940s it was decided that the Faculty of Forestry should remain in Istanbul and the Higher Agricultural School (YZE) in Ankara. Within this framework, the question of which university the Faculty of Forestry should be affiliated to was discussed, and the young faculty members wanted the Faculty of Forestry to be affiliated to Istanbul Technical University, while **Ord. Prof. Dr. Mazhar Diker** (Director General of Forestry at the same time) and **Ord. Prof. Dr. Esat Muhlis Oksal** (Rector of the AYZE), who were influential professors of the time, wanted it to be affiliated to Istanbul University (IU). It was accepted that the Faculty of Forestry would be better affiliated to IU as it had physics, chemistry, biology (PCB:FKB) as well as economics and law in its curriculum[33]. It is also undeniable that these two professors had a great influence on this transition. A similar mistake was made when the Faculty of Forestry was transformed into the Faculty of Forestry at IU Cerrahpaşa [23]. Thus, the institutional identity of the IU Faculty of Forestry (OF) was relegated to the dusty shelves of history overnight. This example should be seen as the second major blow to forestry and the profession, just as it was in the 1940s. The professional and historical ties of the institutions were cut in one night, and this practice was extremely wrong.

**TABLE 2**
**Contributions of foreign forestry and (agricultural) scientists to forestry education [1,15,16,18,20,22,30, 31].**

**A: Those who came from France:**
**1- M. Stheme** and **2-Louis Tassy;** (1856-1866): Opened the forest school in Istanbul, but it received little attention as it taught in French; however, it was closed in 1862 and reopened by Tassy in 1866. **3-Simon;** (1870-1879): Director of the forest school. **4-P.Joseph Saby;** (1925-1926): **a-** Forest ownership in the central organisation of the Forestry General Direction (OGM); **b-** Creation of a department for legal affairs; **c-**Determination of forest boundaries; **ç-** Reforestation reports; **d-** Reports criticising forestry education. **5- Camille Guinet**; (1959-1961): Planning of the Atatürk Arboretum; He was involved in the establishment of the Atatürk Arboretum.
**B-Germans from Germany:**
**1- Prof.Dr.Robert Bernhard**; (1.1926-29; 2.1934-35; 3.1937): He taught silviculture and management. **1-** "Legislation of Turkish Forestry; **2-** History and Tasks" and 3- His contributions to the preparation of the first Forestry Law numbered 3116, which was enacted in 1937. **2- Prof.Dr.Rudolf Pfefferkorn;** (01.10.12.1936-01.08.1939): Lectures on forest production, forest policy and forest road construction; Director of the Institute for Forest Policy and Forestry and Dean of the Faculty of Forestry from 01.03.1937-01.11.1937. **3- Prof.Dr.Karl Fritsche**; (22.10.1936-27.07.1937): Lectures on dendrometry and valuation in the forest; Directorate of the Institute of Forestry and Business Management.**4- Ord.Prof.Dr. Hans Mayer-Wegelin;** (01.01.1937-08.05.1940): Course on the valuation of forest products and business knowledge; Directorate of the Institute for the Valuation of Forest Products. Dean of the Faculty of Forestry in 1937-1939.**a-**Pos Forest Investigation Report.**5-Prof.Dr. Hans Gerd Hildebrandt;** (1972-1973): forest photogrammetry lectures and applications; lectured at (OGM) and Technical Uni of Karadeniz(KTÜ).**6- Prof. Dr. Curt Kosswig**; (1937-1955) Istanbul University(IU); (1969-1980;Erzurum Atatürk U.): General Biology; Genetics; Hydrobiology and Zoology courses. In 1950 he founded and headed the Institute of Hydrobiology at IU, and in 1938 he discovered the Manyas Bird Sanctuary. He told me that he had given Ali, the guard at Manyas, 5 Kuruş for each bird shot, and when he started giving him 25 Kuruş for each bird not shot, Ali stopped shooting birds". He was nicknamed "Turkophile" (Türkophile) and carried out the first bird ringing in Turkey in Manyas. He settled in Emirgan; after Turkey he became Rector of Hamburg University; he was buried with his wife **Dr. Eleonora** in Istanbul's Aşiyan Cemetery; he never forgave the treatment of his Jewish colleagues; he and his wife were not Jews; our generation read his books in the FKB. **7- Prof.Dr.Alfred Heilbronn;** (1933-1962): Lecturer in botany; director of the IU Institute of Pharmacological Botany; learned Turkish and wrote several textbooks on botany and pharmacology. He founded the Botanical Garden of the IU Botany Department in Süleymaniye. He was one of Kosswig's closest friends. **8-Prof. Dr. Friedrich Breusch*; (**1937-1971): He did chemistry courses and applications at FKB; he came to IU when he was the director of the Chemical Institute of the University of Freiburg. He came to the IU when he was the Director of the Institute of Chemistry at the University of Freiburg. II. the Director of the Institute of Chemistry (In our time he gave chemistry lectures, Assoc. Prof. Dr. Emin Ulusoy translated them; He also directed the laboratories himself). **9- Prof. Dr. Neumann,** together with lecturer Rummel, gave German lessons to the students of the Faculty of Forestry.
**C-Those from Austria/Germany:**
**1- Prof.Dr. Erwin Schimitschek*;** (1933-1939): Lecturer in Forest Entomology and Forest Protection; Head of the Chair of Forest Entomology and Forest Protection.
**D-Those from Austria:**
**1- Prof.Dr.Franz Hafner*;** (1937-1938): Lecture on forest management; expertise in the forest management department of the OGM. **2- Prof.Dr. Leo Tschermak (Schermak);** (04.01.1937-04.01.1938): Lectures on ecology

and silviculture. Chair of Forestry; **a-**Participation in the Silviculture Institute. **b-**Selection of suitable sites for eucalyptus afforestation and eucalyptus reforestation in Tarsus. **c-**Forestry studies in accordance with the climate and vegetation of Turkey. **ç-"**Report on the principles of silviculture in Turkey" and **d-"**Report on eucalyptus plantations" are important. **3- Ord.Prof.Dr. Franz Heske; (**He was first invited in 1935 but could not come; then: 1949 and 1951-1955): Forest Manejmant; lectures on forest geography and forestry in dry areas; Director of the Forestry Institute. Lecturer at the Institute for Forest Policy and Forestry Manejmant.**4- Ord.Prof.Dr.Kurt Lohwag;** (1). Visit: 1954-1955; (2). Visit: 1957-1959): Forest phytopathology course; conducted research in the regions of Western Black Sea; Central, Western, Southern Anatolia and Marmara.**5-Prof. Dr. Ralph Gaelzer**; (1988): Landscape planning course; His contributions to landscape architecture are important. **6- Lecturer Wesp** gave German lessons to the students of the Faculty of Forestry.

**E-Faculty members from other countries:**

**1- Prof.Dr.E. Weber (Switzerland);** (31.08.1938-31.07.1942): Geodesy course; while working at Robert College, Istanbul, he took up a position at the Faculty of Forestry, AYZE. **2- Prof. Dr. Alexis Chenchine (Belarus);** (31.08.1939-01.09.1943): Dendrometry; yield information; forest valuation; forest statics and park management. Lectures; Ankara Higher Agricultural Institute, Faculty of Forestry, Directorate of Forest Revenue and Economic Institute. **3- Prof.Dr.Manfred Naeslund; (Sweden);** (17.04.1948-04.05.1948): Worked on the establishment of the Forest Research Institute. **4- Prof.Dr.Michail Prodan; (Romania);** (1961-1962): Course on introduction to biometry. Introductory biometry courses for foresters. Expert on income and biometry.**5-Prof.Dr. K.J. Polakowski; (Poland);** (1971-1972): Park-garden and landscape architecture courses; conducted studies on the separation of the Gallipoli Peninsula as a national park.

***: Prof. Dr. Bruno Huber, Prof. Dr. Erwin Schimitscheck, Prof. Dr. Franz Hafner, Prof. Friedrich Breusch**, who have contributed to the education of the members of our faculty, have lectured at our faculty in the past and made important contributions to Turkish forestry in the field of science and practice. To them, as a sign of gratitude and appreciation from the faculties of natural sciences and forestry, the proposal to the Senate of Istanbul University was unanimously accepted and the "Honorary Doctorate of Science" was awarded to these foreign professors.

**TABLE 3**

**The course and practice schedule (summarised from 15) for the first two years of forestry education in Ankara for the year 1933-34 (for forestry and agricultural education) of the T.C. Ankara Higher Agricultural School (AYZE)**

A) Winter term: 29 September 1933-15 February 1934;
B) Summer term: 15 March 1934-15 June 1934.

**I- INSTITUTE OF FORESTRY:** Director: Ord.Prof.Dr. Franz Heske; Head: Dr. Ali Kemal Bey; Laboratory Assistant: Hakkı Mustafa Bey; Caretaker: Niyazi Mehmet.

***I. Faculty of Natural Sciences:***

(**a) 1-Institute of Physics;** Physics lectures and practical: (Prof. Dr. Zahn: Dr. Sait Ali Osman);

**2-Institute of Chemistry:** Chemistry lectures and practice: (Prof. Dr. Weygand and Dr. Mecit İbrahim Bey);

**3-Institute of Agriculture** (Parts I and II -Forestry-Agriculture-Veterinary students, Prof. Dr. Stüwe. Mathematics (Riyaziyat); Geodesy I and II are done with the help of Engineer Muhittin Bey.

**4- Meteorological Institute**; Meteorologia I. and II. Part Ahmet Tevfik Bey.

**b) Botany and Fauna:**

**1- Botanical (Nebatat) Institute;** General Botany and Microscopic Plant Practice: Prof. Dr. Krause; Dr. Hikmet Ahmet Bey; important topics in the field of forestry and agriculture. Microscopic applications and nebatat observation trips (field trip).

**2- Institute of Zoology:** General Biology and Zoology; Prof. Dr. Woltereck; with the help of Dr. Mithat Ali Bey and Dr. Neu; noteworthy elements in forestry and agriculture.

**3- Biology of the rivers and lakes of Turkey;**

**c) Soils; Mining; Petrography and Palaeontology:** Dr. Şevket Ahmet Bey for students of Forestry and Agriculture (a German teacher should be brought to this chair...).

**II- PREPARATORY BRANCHES FOR FORESTRY AND AGRICULTURE EDUCATION:**

**(a) Main branches:** Forestry; at the Institute of Economics; Prof. Dr. Falke; General Economics I, II and III parts. Applied Economics: Prof. Dr. Falke and Dr. Şevket Raşit Bey (This course lasts 3 semesters).

**4) Forestry Institute;** Relationship of forestry to agriculture and the general welfare of the country, with special reference to Turkey and other subtropical areas; Prof. Dr. Heske; Hunting; Forestry excursions; Dr. Ali Kemal Bey.

**5) Prof. Dr. Gleisberg; Special plant diseases;** Prof. Dr. Hoffmann; Cultivation of meadows and pastures, identification and labelling of grasses. Prof. Dr. Hoffmann; Plant Breeding Practice; Prof. Dr. Gleisberg, Agricultural Practice Dr. Ömer Rüştü.

**6) Institute of Agricultural Chemistry**: Part I and II of the lectures and practicals of Agricultural Chemistry: Prof. Dr. Heide and Halit (Plant use as food: Halit Evliya; Use of animals as food; Mr. Isfendiyar Esat).

**FIGURE 1**
**10 May 1933; an inspection trip to Düzce Fermiköy (right picture: Prof. Acatay top right).**

The professors listed in Table 3 are from the Tharandt Department of Forest Engineering at the Technical University of Dresden, which was one of the most important universities in Europe at the time;(see Table 2) [19]. In this context, when Prof. Dr. Fikret Saatçioğlu retired, Atay [34] emphasised: "Dear Sir, you and most of our esteemed professors who retired before you are not the teachers of a few individuals, but of all foresters of Turkey, of Turkish forestry. It is difficult to put into words the achievements of your generation". Within this framework, a generation has worked hard to ensure that forestry engineering education is carried out in the best conditions and has achieved great success both abroad and in Turkey. The importance of this practice, which is a turning point in our history of higher education and science, and the project initiated by Atatürk to improve the quality of higher education have played a major role in the training of the scientists listed in Table 4 who have received forestry education abroad. In this framework, some information about the life of our teacher, **Prof. Dr. Abdulgafur Acatay**, who is one of those sent abroad and who has made great efforts to bring **"Forest Entomology and Forest Protection"**, one of the most important branches of forestry education, to the present time and whose **120th birthday** will be celebrated in 2023, is presented (Figure 2).

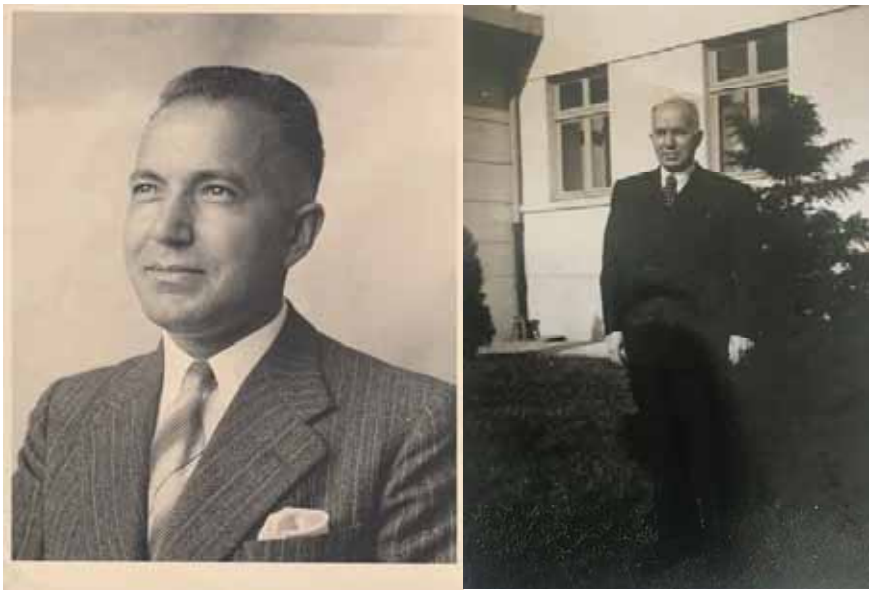


**FIGURE 2**
**The periods of Prof. Acatay's lectureship and professorship.**

## 120TH BIRTHDAY OF PROF. DR. ABDULGAFUR ACATAY

**Prof. Dr. Abdulgafur Acatay**, as an important scientist of the first generation of forestry education, completed both the 8-semester undergraduate course and the doctoral course abroad with great success, thus being one of those who initiated an important process in the establishment of the faculty. The birthday celebrations of Prof. Acatay have been written down [36-39]. My esteemed teacher, Ord. Prof. Dr. Mustafa Asaf Irmak [36], one of the leading minds of this generation, from whose lectures we also benefited greatly and who had a great influence on my inclination towards "ecology", states that his close friendship with Prof. Abdulgafur Acatay began when they were still students at Tharandt and adds.

In this small but spiritually and intellectually rich academic environment, Prof. Abdulgafur Acatay (then a young and very handsome student), with his shy, quiet character, dignified demeanour and bright black eyes that smiled from within, made friends, and his deep understanding and diligence earned him the admiration of his professors. He was one of those who made a name for Turkishness in a foreign land. A friendship that began back then in Tharandt was to last a lifetime, as it turned out. If only you knew how many years have passed in such a short time. Professor Abdulgafur Acatay was born on 10 July 1903 in a charming house in the vineyards of Denizli. Professor Abdulgafur began his long journey of reading with his first step at Nümune School in Denizli. Then he could have knocked on the door of any vocational school with his graduation certificate from Denizli Idad. But nature and especially the plant world lured him at that time with its mysteries to be solved and regions to be illuminated. So he entered the College of Forestry in the autumn of 1923. Here he stood out as a very capable student, and in 1926 he graduated with honours and became a "forest scientist".

While Prof. Acatay was a forestry official first in Tavas and then in Bozüyük Yürce-Bürmeceler in the first phase of his career, **Prof. Robert Bernhard,** the former Director General of Forestry of the Kingdom of Prussia, Saxony, was invited to Turkey in 1926 to serve as an advisor to the Ministry of Agriculture in the framework of the preliminary studies for the establishment of a modern Turkish forestry. Prof. Bernhard visited Turkey three times and worked in Turkey for a total of 6.5 years (17; see Table 2). Prof. Bernhard, who spent the longest time in Turkey among the foreign experts and made a significant contribution to the development of Turkish forestry, earned important merits in the fields of ***a) Reorganisation of forestry organisation; b) Aforestation; c) Protection of forests; d) Regulation of forestry work and d) Development of forestry education.*** Prof. Bernhard's work on these issues took the form of conferences, draft laws and lectures for students of forestry and agriculture at the Ankara College of Agriculture. Among these merits, the most important are his contribution to the preparation of the book "Legislation, History and Duties of Turkish Forestry" and the Forestry Law No. 3116, which was enacted in 1937. This law is significant in that it is the first forestry law in Turkey. Prof. Dr. Bernhard, who had rendered great services to Turkish forestry, wrote a letter to **Atatürk** before his departure, trying to implement his proposals and efforts for the development of Turkish forestry. When **Prof. Dr. Bernhard** came to Turkey in 1926 to develop Turkish forestry, Prof. Acatay accompanied him to Bozöyük and its surroundings and took over his assistance. Prof. Bernard, who was very pleased with this situation, suggested to Prof. Acatay to send young and hard-working forestry engineers like you abroad to study and even to obtain a doctorate. Within this framework, Prof. Acatay was assigned to the Ankara Forest Tree School in 1930 on his recommendation. While working here, he was personally involved in the establishment of the **Atatürk Forest Farm**. What is important is what Prof. Acatay told Prof. Dr. Yurdakul Yurdakul in this context [40]: *''-The intensive work on the farm began to bear fruit after a few years. Pasteurised milk, yoghurt, cheese, butter and very high quality, healthily grown vegetables and fruits were made available to the public. Ankara Municipality opened two shops in the city to sell these products and started offering them at very reasonable prices. Ankara, which was poor in trees, greened up thanks to these efforts, and all the population's needs for seedlings and seeds were met from here in the highest quality. In the past, when we spoke of roses in our country, we meant the pink roses grown in the regions of Isparta, Burdur and Denizli, which are now cultivated only for their oil. Roses of other colours were unknown to our people. When Atatürk had the forest farm built, he wanted to open a rose nursery and have the colourful roses he had seen in Europe produced here. After the preparations were made, a wagon full of different coloured rose seedlings was brought from Germany and Holland. We multiplied them and sold them at very reasonable prices to embassies, our people, parks and gardens. Most of the different coloured roses that we see almost everywhere in our country today are descended from the products that Atatürk brought at that time and which were multiplied and distributed by us."* Prof. Acatay compiled his studies on "*Rose and Rose Oil*" in a book and also studied the pests of *Rosa domesticana* [41].

**TABLE 4**
**Scientists representing important milestones of forestry education in Turkey and the country where they were educated abroad; university and PhD topics (based on 19 and 21)*.**

**A- Faculty of Forestry, University of Tübingen, Germany:**
**1- MAZHAR DİKER** (1899-1952): Management course at the School of Forestry. Later rector of the school.
**B- Royal Prussian Forestry Academy in Eberswald, Germany:**
**1-ESAT MUHLİS OKSAL** (1888-1970): Lecturer in silviculture. Lecturer at the College of Forestry. Rectorate of YZE
**C- Those who have completed their PhD after a Bachelor's degree in Forestry at TU Tharandt Faculty of Forestry in Dresden, Germany:**
**1-M.ASAF IRMAK** (1905-1996); PhD: Contribution to the ecology of fir; "Finish: 1933; With whom: Prof. Krauss; Grade: Very good. Head of the Department of Forest Ecology; holds the first associate professorship in the Faculty of Forestry. **2- ABDULGAFUR ACATAY** (1903-1993): Dissertation: "Studies on the quantity and quality of seed set in different crown parts of indigenous forest trees": executed: 1938; with: Prof. Bruno Huber/Note: Good: senior assistant at the Higher Agricultural School, head of the Department of Forest Entomology and Conservation; IU OF Senator. **3- HAYRETTİN KAYACIK** (1911-2001): Dissertation: "Fundamentals of Reforestation in the Mediterranean Area with Special Reference to Italy and Turkey"; Completed: 1941; To whom: Prof.Rubner; Grade: Good; Lecture on the life, internal and external structures of forest trees; Forest botany; Foundation of the Atatürk Arboretum. **4- SAVNİ HUŞ** (1911-1996): Dissertation: "Forest significance of Liquidambar orientalis Mill. and chemical study of its balsam, Turkish Storax": End: 1940;To whom: Prof. Wienhaus;Degree: Very good; Chair of Forest Plant Utilisation; Forest Chemistry and Hunting Science. **5- REFİK ERDEM** (1912-1998):Dissertation:" Experiments on the effect of touch poisons on the caterpillars of the pine processionary moth as a basis for controlling the processionary moth inTurkey";Degree:1940/with whom: Prof.Prell/ Diploma: Fair; Chair of Forest Entomology and Conservation; 1962-64 IU OF Dean.**6- KEMAL SAVAŞ;** Thesis: "Forest grazing in Turkey, its present extent and its future economic as well as legal regulation"; Degree: 1940: With whom:Prof.Mantel; Degree: Very good; Worked in different departments of Forest Organisation. **7- ALİ TOPÇUOĞLU;** Dissertation: The distribution of increment on the stem length of trees".Finish:1940;With: Prof.Bruno Huber;Good; Worked in different departments of forest organisation.
**D- Department of Forestry, Faculty of Economics, Ludwigs-Maximilians-Universität Munich;**
**1- Ş.NURİ İLKMEN** (1903-1983); Dissertation; "The Protection Forest in Turkey" End: 1938; ;With whom Prof.Dietrich/Diploma: Very good; General Economics and Agricultural Policy; 1956-58 IU Faculty of Forestry. Dean's office. **2- FİKRET SAATÇİOĞLU** (1910-1983); Bachelor's degree: 1934, very good; PhD: "Mutual Growth Relationships in Spruce and Beech Mixed Stand"; Degree: 1935; Prof.Fabricius/Pekiyi: YZE: 1935 Senior Assistant; Director of the Department of Silviculture; 1948 Dean of YZE Faculty of Forestry. Dean; Ministry of Forestry in the government of Prof. Sadi Irmak;**3- ADNAN BERKEL** (1908-1988); Dissertation; "Studies On Issues Of Yield Regulation In The Forests Of Turkey" 1935; With whom: Prof. Schüpfer: Director of the Institute of Forest Crop Valorisation, Faculty of Forestry, YZE; Director of the Chair of Forest Crop Valorisation, Faculty of Forestry, YZE; Dean of OF, IU, 1958-59. **4-FEHİM FIRAT** (1908-1980); School of Forestry, Nancy / Faculty of Forestry, Dresden TUTharandt: PhD with: Prof.Rohl/Pekiyi; Director of the Chair of Forestry and Business Administration; Dean of the Faculty of Forestry 1952-54; Rector of IU 1955.
**E- Department of Forestry, University of Natural Resources and Applied Life Sciences Vienna**
**1- SELAHATTİN INAL** (1909-1996); Thesis: 'The relationship between tannin content in oak bark and growth environment."End: 1941; Degree: Very Good; Director of the Department of Forest Policy; 1960-62 Dean of IU OF; Acting Rector of IU; Minister of Forestry in the Nihat Erim government. **2- FAİK TAVŞANOĞLU** (1908-2001); Dissertation; "Rational transport of water channels"; graduated: 1937; Director of the Department of Forestry and Transport; Dean of IU OF 1951-52.
**F- 2 years internship at Ecole Nationale Eaux et Forest in Nancy, France/ TU in Munich, Germany.**
**1- KEMAL ERKİN** (1910-1989): Internship at the Department of Surveying at TU Munich; Dissertation. "Volume, Shape Mode and Generally Proceeds Investigations on the Sarchams of Seben District"; Finish: 1948; in Turkey; Geodesy course; 2 times Dean of IU OF in 1966 and 1968.

*) **Prof. Dr. İsmail Eraslan** (1917-2007), who studied and obtained his PhD in Turkey, should be included in this list (see [35].

Irmak [36], describing his time with Prof. Acatay [42] in Tharandt, Germany, added: *"-he emphasised the fact that he served the country with his unique individual achievements and stated that this was appreciated by the higher authorities." According to Irmak (1963), the reward for these successful achievements was not delayed: In the spring of 1931, he passed the examination he had taken to broaden and improve his education and was sent to Germany. He spent the first year wrestling with the difficult German language, but Abdulgafur hoca knew how to overcome it, as he did with every obstacle. Thus the doors of German universities were opened to him. In 1932, as he walked through the remote streets of Tharandt with his bag in hand, he entered the threshold of the world-famous University of Forestry. As the undergraduate study guide shows, he had passed the required exams in all courses and paved his way to the finals. Here he proved to be a truly brilliant student, and in his pocket was the diploma he had received in February 1936. Prof. Dr. Bruno Huber had his eye on the*

*diligent student and encouraged him to do a doctorate. The first studies were started, but the General Directorate of Forestry (OGM) was impatient: he had completed his studies; what was this man still doing there? In great modesty and silence, without even daring to mention his work, this man worked feverishly on a doctoral thesis that would later become very successful. There was no time left to finish it, and in March 1937 he was appointed senior assistant at the Faculty of Forestry of the Higher Agricultural School. His thesis at Tharandt was also of interest: "The Biology of Tree Tops", which had not yet been solved, dealt with the question of "whether the generations that develop from the specific seeds differ at the different heights of the trunk".*

After completing his diploma studies, he went to Tharandt again, took advantage of the summer holidays in 1937 and completed his doctoral thesis entitled *"Studies on the quantity and quality of seed set in various crown parts of native forest trees"*. On 22 November 1937, he completed a two-hour doctoral examination with an excellent result and received the title *"Engineer-Doctor of Forestry"* and then returned to his beloved country [36,38]. After his return home, Prof. Acatay became an assistant to Prof. Schimitschek at the Institute of Forest Entomology, Forest Conservation and Wildlife Science and carried out lectures and applications together with him, earning the recognition of Schimitschek [37] through his diligence and exceptional human quality. With the return of Prof. Schimitschek in 1939, Prof. Acatay took over as director of the Institute. He completed his habilitation in 1941 and his professorship in 1946 (Figure 3). In fact, **Prof.Dr.Dr.Dr.h.c. Bruno Huber**, the most important botany professor in Europe at the time and director of the Institute of Forest Botany at LMU-Munich, with whom he had completed his doctorate, that he take up a position in the Botany Chair at the IU Faculty of Forestry. At that time, however, Ord. Prof. Dr. Esat Muhlis Oksal held this chair, and Prof. Acatay, who knew the difficulties of working with him, considered such a choice more appropriate. Schimitschek [37] notes that he achieved great success in three disciplines: forest entomology, forest conservation and botany. This is because Prof. Acatay was also very well educated in botany; for example, Selik [38] had first heard of his finding during Prof. Huber's lectures in Munich in 1957 that the female floral dichasias (flower stalks below the apical flower are opposite and more or less equal in length; two lateral branches are at the same height) in members of the Fagaceae family actually have many flowers (more than 2). Huber's lectures in Munich in 1957, and we listened with pleasure to his words about Acatay, in which he addressed the class and added: *"-I always think and remember with pleasure this determination of my former student, Mr. Selik's teacher Acatay"* [43-45]. Prof. Huber never forgot this former student, whom he held in high esteem, and always kept in touch with him. In the meantime, his doctoral thesis was cited in textbooks in Germany and the importance of his work became clear. The methodology and experimental results of this work were followed up and evaluated in the post-war period, despite the long World War II and the various confusions and irregularities it caused, and in the last work of Prof. Ernst Rohmeder, the famous authority on the knowledge of seeds of forest trees, "Saatgut in der Forstwirtschaft, Paul-Parey, Hamburg-Berlin, 1972, p. 273", a large space was devoted to these studies and the latest developments in this field were discussed. The fact that the scientific work of a Turkish forest engineer (Dr. Abdulgafur Acatay) is so influential is a situation to be very proud of on behalf of forestry [46].

**FIGURE 3**
**14.11.1953 Examination for a Habilitationexaminas of the Aegean Faculty of Agriculture in Izmir, with jury members and candidates for a lectureship (Prof. Acatay on the left).**

Our esteemed teacher, Prof. Acatay, always held progressive and modern views in solving many forestry problems, which is why he often attracted attention in solving the country's forestry problems [47-62]. On-site investigations and scientific travel were part of Prof Acatay's lifestyle. He had extensive knowledge and experience in national and international forestry issues. His knowledge was called upon at every opportunity to solve various forestry problems. In particular, his ability to speak German without accent like a German and his knowledge of German literature provided him with an important privilege and a great contribution to Turkish forestry. On the one hand, he expanded the scientific Turkish catalogue of the department of which he was the head and pioneered the Turkification of many terms. Many new concepts of entomology and forest conservation were introduced into the literature thanks to his work[48-103]. The students who were in Prof. Acatay's group, especially during fieldwork, got to know many professional problems both in Turkey and abroad thanks to him. It is well known that the students in his group acquired extensive knowledge of both forestry and general life issues during field studies and scientific study trips (excursions). These excursions were also very important events for him (Figure 11). He also attached a lot of importance to them.

In addition, Prof. Acatay conducted numerous scientific studies to educate the public and those interested in current issues [53,60, 68,84, 97,104-106]. Thus, in 1942, Prof. Acatay successfully initiated and led the fight against the pine beetle on the Istanbul islands and especially in the Büyükada pine forests: within this framework, Prof. Dr. Abdulgafur Acatay was appointed by decision of 31. May 1943 and number 451 to investigate the pine beetle damage in the Büyükada pine forests, to take the necessary measures and to organise and complete the fight in agreement between the Istanbul Municipality and the General Directorate of Forestry [78,102,107,108]. As in forestry, he also served as a jury member in numerous examinations for habilitation in agriculture.

As a human being, Prof. Acatay was a mature, humble, unpretentious and extremely loyal to the smallest friendship and loyalty, with a soft nature that never hurt anyone, compassionate and understanding towards his students, in short, a complete human perfect. Prof. Acatay sometimes asked very interesting and exciting questions during lectures and scientific excursions: for example, the original name of the plant is chrysanthemum (Chryssianthemus ssp.); however, in our country the plant is called chrysanthemum in Turkish because of its phenology (the science of awakening, budding, flowering, etc. of plants depending on the climate). 48 years ago, during a conversation in the garden of the Faculty of Forestry, our late teacher Prof. Acatay said;

*"-Young people, I guess: Why do chrysanthemums explode in November"?* When we made neither a good nor a bad sound, he explained the scientific answer: *-"Chrysanthemum is a short-day plant; it cannot form flower buds on days longer than 12 hours (in summer), and the most favourable time for turkey is November. During the flowering period, the night temperature must be below +15 °C; therefore, the chrysanthemum is a winter plant that tolerates cold down to -20 °C".*

Prof. Acatay had an extensive knowledge of both forest trees and herbaceous plants, as well as significant knowledge of forest pests and forest protection. Especially his research on insect pests in the forest is of great importance. In this context, his ***"Key Book for Forest Insects***" is one of the most important scientific studies on this subject [70,77,79,91,92,109]. He also described a cedar pest and the taxon *Phloeosinus acatayi* Schedl 1958 (= *Phloeosinus cedri* subsp. *acatayi*) was dedicated to him; now *Eriophes cedri* [64,73,88]. Schimitschek (1973) states that he discovered the cedar pests *Buprestis cupressi, Chrysobothris solieri, Magdalis violacea* and the new species *Phloeosinus acatayi* and *Crypturgus cinereus* as well as the cedar pest *Acalla undulana*, whose biology he particularly emphasised[52,75,110]. He also discovered a new species of cedar pest called *Barbara osmana* Obr. and pointed out its biology. He also studied the biology of the scale weevil, *Acantomytillus cedricola* and *Megastigmus schimitscheki* (37). He also conducted studies on important poplar pests. He studied more than 60 insect species, which he identified in the Belgrade Forest in Istanbul[44,81,111-116]. He identified *Phoracantha semipunctata* as the most common pest of Eucalyptus [111,116] and the top shoot pest of Pholeosinus [52,116]. He also carried out important research on the giant cambium beetle, *Dendroctonus micans*, which causes massive spruce dieback, especially in the spruce forests of the eastern Black Sea, as well as on hazelnut pests, *Liquidambar orientalis*- and pistachio pests [66,94,117, 118]. Prof. Acatay was the first to establish the existence of Dutch elm disease in Turkey in 1940 and the first to publish on the subject [41,47,63,114]. He published on semi-parasitic plants such as mistletoe (*Viscum album)* and Cuscuta; he was also interested in other phytopathological problems of forestry in our country and did research on forest diseases caused by fungi [80,115,119]

As noted by Selik (1973), he made numerous innovations and contributions to forestry in the fields of forest botany, applied botany, dendrology, forest phytopathology and hunting [41,43,68, 69,73,74,82,86,115,117,118,120-123]As part of his botanical studies, Prof. Acatay was the first to identify *Betula medvedevi*, an endemic species, in

the eastern Black Sea region of Turkey [64,73,74,75,110]. His studies on *Alnus orientalis* and cedars in the Bozdağ region were very important [73]. Later, he expanded our knowledge of *Liquidambar orientalis*, a tertiary relict in southwestern Anatolia, with new morphological and distributional determinations[117] and described a fungal variety of the same species, var. *suberosa* on naturally growing shoots of the same species. He also wrote an introductory publication on *Populus euphratica*, an intermediate poplar species [120]. In addition, he was also credited for clarifying and concluding the controversial question of the southern limit of *Pinus silvestris* L. in Turkey and thus in the Northern Hemisphere. Thus, the southern limit of yellow pine in western Anatolia was assumed to pass through the Murat Mountains around Kütahya [81,86,]. He has also identified and described a new cultivar, *Pinus nigra* var *pyramidata* [81] with a pyramidal habit, found near Tavşanlı and made an important contribution to the dendrological literature of our country and the world [52,82,87,123]. Today, *Pinus nigra* subsp. *Pallasiana* var *pyramidata* (Acatay) Yaltırık; is called "Ehrami pine". The distribution area of the variety is 25 km from Tavşanlı and 2 km from Vakıf village. It is sparsely distributed in a higher area southeast of the village on an area of about 250 ha. The stand, mixed with larches, oaks, junipers, etc., is located at an altitude of 1000-1100 m. It spreads on both sides of the stream in a south-north direction, especially on the west side of the stream. The soil is very stony and the bedrock is limestone [82,123]. The OGM named this forest in Tavşanlı after him and put up a sign, showing a great example of gratitude. I was very proud to see this sign during a scientific trip to this region in the 1990s; I do not know if it is still there today. In his continuous studies, especially in his research on *Cedrus libani* in Bozdağ near Denizli, Acatay determined the morphological differences in cedar cones and found that the change in the colour of the crown from green to silvery is a phenomenon that occurs from about 30 years of age.

Prof. Acatay also made many innovations in the field of forest protection, another field in which he carried out important scientific research: for example, he also dealt with forest fires, the creation of safety belts and storm surges [58,62,124]. He also conducted research on bird conservation [106]. In addition, walnut and walnut cultivation [68] and the ***''Textbook on Forest Conservation''*** [59,89,125] are also very important scientific works [37]. Apart from this, it was found that grazing of domestic animals in the forest and especially the constant roaming of hair goats in the forest and providing food were among the most important forest destruction factors [67,71,72,76,103,126]. According to the study of [127], there were 15 315 000 hair goats in our country in 1966. The grazing of most of these goats in the forests on the one hand makes it impossible to increase the existing forest area and on the other hand largely prevents the creation of new forest areas. In the meantime, it is emphasised that the damage caused by the owners by cutting down and pruning the trees in the forests and damaging the youth to feed the hair goats cannot be ignored. It is also clear that these problems cannot be solved only by taking action under legal regulations or through the efforts of forest managers. When goats graze in the afforestation areas for red pines, black pines and cedars, almost all the seedlings planted in a short period of three years are destroyed. It has been emphasised that the problem is severe in natural regeneration areas and goats have been shown to play a negative role as a disturbance factor for forestry. In the natural regeneration areas of cedar, it has been experimentally found that more than 80 % of the one-year-old saplings are destroyed. It has therefore been proven that it is impossible to ensure the sustainability of forest ecosystems, i.e. to protect forests by the presence of goats [59,67,72,76,126,127].

As a person, Prof. Acatay was mature, modest, unpretentious and extremely loyal to the point of the smallest friendship and loyalty, gentle in his manner that hurt no one, kind and understanding towards his students, in short, a complete human being. This year we celebrate the 120th birthday of our late teacher, and the successful and long years of teaching and research he left us will always be with us. Prof. Dr. Erwin Schimitschek *''-Do you know what my Turkish students called me during the interview?* Knowing how agile and intelligent the Turks were, and without him saying it, he couldn't help laughing when I told him that they might have called you - *"Blitz" and said, yes, they called me "Şimşek".... I still remember that name as a great beauty''*Schimitschek, who had risen to the rank of professor at the same school, was invited to the Higher Agricultural Institute in Ankara, Faculty of Forestry, to head the Institute for Forest Protection and Entomology and to give lectures. Between 10.03.1937 and 01.01.1939, Schimitschek taught the subjects of forest entomology and forest protection, and at times also wildlife science and hunting. During his stay in Turkey, Schimitschek travelled through southern, northern and central Anatolia and carried out studies that formed the basis for forest entomology in Turkey and wrote valuable publications on forest protection and forest entomology. Some were translated into Turkish by Prof. Acatay. Prof. Schimitschek, who was later President of the Institute of Forest Zoology at Göttingen University, came to our country in 1959 at the invitation of the IU Faculty of Forestry to give lectures and gave three conferences. On the recommendation of the Faculty of Forestry, Schimitschek was awarded the title of *"Honorary Doctor"* by the Senate of Istanbul University in

1974. In an institute consisting of four walls and two cupboards, two scientists embarked on an arduous but honourable experiment and thus founded and ran the "Institute of Forest Entomology and Forest Protection ", which is extremely important for forestry, and ensured that it still exists today. Prof. Acatay explained that at that time there were no books, microscopes or even magnifying glasses at the chair. In such a start-up phase, he translated Prof. Schimidtschek's works into Turkish and even worked as his assistant when needed. However, in this working atmosphere, all the members of this founding staff of forestry who were trained abroad under the guidance of Atatürk's genius were determined; all the members of this staff who started from sound knowledge, whose determination to work with an excellent perseverance and untiring energy was above all, and who did not hesitate to work for the forestry education of Turkey and the teaching of the profession, achieved great success. And by putting stone upon stone, a solid structure was built. Prof. Abdulgafur and his professional work at the Faculty is the common story of those who pioneered the establishment of a scientific institution. After Prof. Shimitschek's return in 1939, Prof. Acatay took over the chair and became an associate lecturer *"Harmful forest insects in the Istanbul area and especially in the Belgrade forest, their control and impact on management"*. At that time, Prof. Abdulgafur Acatay made one of the most important decisions of his life: he had chosen his future partner... With this choice, Prof. Acatay proved once again that he is a man of taste, far-sighted and precise in his decisions. He married Mrs. Dirayet, who was one of the first teachers from Atatürk's time and who, as he always proudly told, Atatürk came to her school as headmistress *"Başöğretmen"*. Teacher Dirayet supported him at all times and in all places and created a harmonious, quiet and peaceful home life for him, which played a great role in the success of his later work. He had three daughters and six grandchildren.

The scientific publications we have listed in this survey clearly show the high level of academic success of our esteemed teacher in his many years of teaching. The sincere love and respect for his personality is an element that should always be remembered. We are sure to echo the thoughts and opinions of countless students, friends and colleagues who love and respect him, and we wish him, our beloved Abdulgafur Hoca, God's abundant forgiveness. We want him to keep the banner of forest entomology and conservation that he inaugurated in good hands. His important contributions to forestry in the country will continue to be with us through his books and publications.

## REFERENCES


[1] Widmann, H. (1973). *Exil und Bildungshilfe*. Die deutsch sprachige akademische Emigration in die Türkei nach 1933. Frankfurt /M,; Peter Lang International Publisher.

[2] Neumark, F. (1980). Neumark, F. (1980). Refuge on the Bosporus. ,German Scholars, Politicans and Artists in Emigration 1933–1953. Knecht, Frankfurt am Main;1980.

[3] Hirsh, E.E. (1982). From the Times of the Kaiser through the Weimarer Republic to the Atatürks Country,an outdated Autobiography. J. Schweitzer Verlag, München; 1982

[4] Schwartz, P. (1995). *Notgemeinschaft:* Zur Emigrationdeutscher Wissenschaftler nach 1933 in der Türkei. Paperback, Helge Peukert (Herausgeber, Vorwort). Marburg Metropolis-Verlag. 1995.

[5] Kazancigil, A., Ortaylı, İ. & Tanyeli, U. (2000). Turkey´s Foreigners, Yapı Kredi Yayınları. *Üç Aylık Düsünce Dergisi,23,* 119-132.

[6] Dalaman, C. (2001). Turkey in its Modernization Phase as Emigration Country for German Exils. PhD-work, Sozialwissen-schaftliche Fakultät-Freie Universität Berlin.

[7] Kayadibi, F. (2006).Developing of Science and Education during the Ataturk- Period. *İstanbul Üniversitesi İlahiyat Fakültesi Dergisi 13*: 1-2.

[8] Özata, S. (2007). *Atatürk ,Science and Universities*: TUBİTAK Popüler Bilim Kitapları.-Ankara

[9] Çam, H. (2012). Migration of German Scientists between Two World Wars. Istanbul University Social Sciences Institute, PhD. Thesis -Istanbul 2012.

[10] Erdem, A.R. (2012).University reform under the leadership of Atatürk: The turning Point in the History of Higher Education and Science. *BELGİ.Vol 4,2*,376-388.

[11] Namal, Y. (2012). Contributions of Foreign Scientists to the Higher Education Between 1933–1950 in Turkey.*Journal of Higher Education and Science 2 (1)4,*14-19. DOI: 10.5961/jhes.2012.028.

[12] Meydan, S. (2015). Turkish Enlightment. Atatürks Smart Projects.337,1-1368. İnkilap - Press, İstanbul

[13] Meydan, S. (2022). Key to Recent History. İnkilap -Press, , İstanbul, 344,65-71.

[14] Koz, M. (2019). Famous Scientest.Refuges in Istanbul.TAVAK 295,174-187.

[15] Şen, F. (2019). Refuges in İstanbul,those who make life better in Germany later. TAVAK issue No.295

[16] Şarman ,Y. (2021). German experts working in Ankara during the university reform process 1933,and their Studies1933. IKSAD Publishing House.ISBN: 978-625-8007-88-6.

[17] Bernhard, R. (1935).Legislation,History and Duties of Forestery in Turkey.Special Report from Higher Agriculture Institute.Ankara-Turkey.
[18] Özdönmez, M. & Ekizoğlu, A. (1994). Foreign experts,contributed to Republican Forestry Period. Journal of İstanbul University-Forestry Faculty *B,44, 1-2,* 13-28.
[19] Özdönmez, M., Akesen, A. & Ekizoğlu, A. (2000). First Generation Forestry-Faculty Members of University Istanbul(1933-1960) Journal of Istanbul University-Forestry Faculty B.50,1-22.
[20] Dursun, S. (2010). A Comparative Study of French and German Impacts on Ottoman-Turkish Forestry (1856-1937).Lecture given on 06.07.2010.urn.nbn.de.gbv.547-2011 00318.
[21] Voss, W.& Musiol, A. (2015). Biographical Encyclopedia of Earlier Doctorates at the TU-Dresden (1900–1945). Lienert M. Archive of the Technical University of Dresden. (https://www.ua.tu-dresden.de/PDFs/voss2015pf.pdf )1237
[22] Gümüş, C. (2018). Turkish Forest Revolution.Newsletter of theTurkish Forester´s Union-Ankara, 2018-324www.ormancilardernegi.org
[23] Sevgi, O. (2021). Periodical Name Changes in the Higher Education Forestry Institutions of Turkey *Journal of Higher Education (Turkey), 11(1):* 1–9
[24] Bahadır, O. (2007). Turkish Academy of Sciences Publications, Yeni Reform Printing House, Ankara 2007, s.8-9.
[25] Türk, F, Çınar, S. (2013). Scientific Relations Between *Turkey and Germany: Turkish-German Universities. Akademik* Bakış 45(7): 13, 1-11.
[26] Möckelmann, R. (2016). Second Homeland Turkey, Ernst Reuter´s Ankara Years.Turkish Business Bank.Cultural Publications-Istanbul, September. 54-55
[27] Yalçın, K. (2019). *Haymatlos.*Turkish Business Bank-Cultural Publications/Istanbul.2249.64-70
[28] Paksoy, N. (2022). A Forgotten Savior.Philipp Schwartz. Dokumentarische Geschichte.Special Issue.May 15, 2022
[29] Kiziroğlu, I. (2022) Introduction to University Life.OSTIM Technical Publication.Enlarged 2nd Edition
[30] Kutluk, H. (1957) How were the French Forestres brought to Turkey?By whose request the Forest School was opened?For how much time? Turkish Foresters Association .Special Issue 18-21, Ankara.
[31] Eryılmaz, A.Y. (2008). Forestry Teaching in the Historical Process of Development, 3rd National Forestry Congress (Forestry Education in Turkey in its 150th Year), 20-22 March 2008. *Turkish Foresters Association*, pp.31-44, Ankara.
[32] Kreiser, K. & Neumann, C. K. (2009). Kleine Geschichte der Türkei. Ditzingen: Reklam Verlag.
[33] İlkmen, Ş.N. (1974). Speeches at the ceremony for the 1973 retired faculty members of I.U. Faculty of Forestry (Prof.Dr. Şeref Nuri İlkmen, Prof.Dr. Abdulgaffar Acatay, Prof.Dr. Selahattin İnal) (IU Faculty of Forestry in 1973, retired), Sermet Matbaası, Istanbul.
[34] Atay, I.(1981).Our Distinguished Professor Prof. Dr. Fikret Saatçioğlu'(Science and Life and Works). *I.U.Faculty of Forestry Journal Series* A, Volume 31, Issue 1: 1-10.
*[35]*Eler, Ü & Asan, Ü.(2007). Eraslan is 90 years old.. *Journal of İstanbul University Faculty of Forestry Series B, Issue 2:* 37-44.
[36] Irmak, A. (1963). Prof.Dr. Gafur Acatay is 60.years old. *Journal of İstanbul University Faculty of Forestry B, XIII, No. 1:* 118-122.
[37] Schimitschek, E. (1973). Professor Dr. Abdulgafur Acatay is 70 years old. Rundschau. *Journal of Pest Science ngskunde XLVI:* 109-110.
*[38]*Selik, M. (1973). Prof. Dr. Abdulgafur Acatay is 70 years old . *Journal of İstanbul University Faculty of Forestry B: XXIII, 1, 1-2*
[39] Kiziroğlu, İ. *(1994)* Acatay Abdulgafur-In Memoriam. *Journal of Pest Science 67* (5)(1994): 111.
[40] Yurdakul, Y. (2013). *Unpublished memories of Atatürk*(10.Auflage). Truva Kitapevi-Istanbul
[41] Acatay, A. (1969). *Rose and rose oil.* Özaydın-Press İstanbul.
[42] Acatay, A (1965) Prof.Dr. Asaf Irmak is 60 years old. *Journal of İstanbul University Faculty of Forestry, B, XV (2) :* 158 - 164.
[43] Acatay, A. (1940). How to get good seed? *Forestry and Hunting,12,4;* 122 - 125.
[44] Acatay, A. (1941).Economic Importance and Establishment Technique of Poplars. *Forestry and Hunting 6-12,* 99-103.
[45] Acatay, A. (1941 – 1942).The Denial of a Tree Generation. Yedigün Journal, 1,452,13.
[46] Rohmeder, E. (1972). *Seeds in Forestry.* Paul-Parey-Printing, Hamburg - Berlin, 1972, 273-282.
[47] Acatay, A. (1943).Research on Drying Trees around Istanbul. *Forest and Hunting,1,*18 - 24.
[48] Acatay, A. (1945). Pest Insects. Economic Walk. *Forest Special Issue, 6 (6):* 38 – 40
[49] Acatay, A. (1951). Growing Willow in Suitable Field Borders,One of the Measures to Prevent Forest Destruction. *Journal of İstanbul University Faculty of Forestry B, 1 (1)* : 1 - 4

[50] Acatay, A. & Huş, S.(1954).Investigation and Proposals about the Hunting Area that will Establish a Facility in Uludağ. *Journal of İstanbul University Faculty of Forestry B, 4(1)*.90 - 95.
[51] Acatay, A (1958). Sampling and Transportation of Insect Infested and Diseased Samples. *Journal of İstanbul University Faculty of Forestry B, 8 (2) :* 16 - 24
[52] Acatay, A. (1958). Triebfrass von *Phloeosinus armatus* Reitter und *Phloeosinus bicolor* Brull. *Anz. für Schädlingskunde XXXI (9)* : 129 - 130.
[53] Acatay, A,Saatcioglu F,Hus S,Eraslan I(1960d). Investigations on the Differentiation and Organization of Qualifications that can be an Exemplary State Enterprise in Turkey. *Istanbul University,Forestry Faculty Journal B, 10 (2)* ,44 - 70.
[54] Acatay, A. (1962). Atomic Warfare against some Insects. *Journal of İstanbul University Faculty of Forestry B, 12 (2),* 17 – 21
[55] Acatay, A., Pamay,A & Kalıpsız, A. (1962). Süleymaniye Dişbudak-Forest,thoughts about its Development and Restoration. *Journal of İstanbul University Faculty of Forestry B, 12 (2)* ,38 - 54.
[56] Acatay A (1967 – 1968) *Pinus nigra* var. *pyramidata* und eine interessante Beobachtung. *Mitteilungen der Deutschen Dendrologischen Gesellschaft Nr. 63*:54-55.
[57] Acatay, A. (1968). Dendroctonus micans Kug. as a new spruce seedling in Turkey. *Istanbul University,Forestry Faculty Journal, A, XVIII (1):* 18 - 36..
[58] Acatay, A. & Gülen, İ.(1966). Comparison of German and Austrian State Forest Enterprises in terms of Productivity and Work Volume of Forest Enterprises. *Istanbul University,Forestry Faculty Journal B, XVI (2)* :17 - 25.
[59] Acatay, A. (1966). *Forest Protection.* Faculties Matbaası, IstanbuL. XI + 308 pp. (Modified and expanded Second Edition).
[60] Acatay, A. (1970b). Oak Monoculture in the Thrace Region, the Necessity of its Conversion to Grove and Change. *Journal of Forestry Research Institute, Ankara, 16 (2)*:3 - 12
[61] Acatay, A. (1971). Über das Auftreten einiger Forstschädlinge in der Türkei. *Journal of Pest Science,, XLIV (11) :* 161 - 165.
[62] Acatay, A. & Gülen, İ. (1971c). Wind damages in Turkish forests. *J. of İ. U. Faculty of Forestry A, XXI (2)*: 1 - 20.
[63] Acatay, A. (1942). Über das Auftreten von Forstschädlingen in der Türkei. *Centralblatt für das gesamte Forstwesen, Heft 1, 69. Jahrgang*: 1 - 4.
[64] Acatay, A. (1943). *Eriophyes cedri* Acatay. *Ankara Journal of the Higher Agricultural Institute*, Issue *1*: 419 - 426
[65] Acatay, A. (1944).Flower Formation in Plants. *Forest and Hunting, 7,*257 - 260
[66] Acatay, A. (1945). Insects infesting Hazelnut Trees. *Ankara Journal of the Higher Agricultural Institute*, Issue 1 (7), Year 2, Volume 4: 266 - 278. (Translated from Prof. Dr. Erwin Schimitschek).
[67] Acatay, A. (1945) Enemy of the Forest, the barbel*. Ulus Newspaper, 4.1.1945*, p. 5.
[68] Acatay, A. (1945). Cultivation of Walnut Tree. *Forest and Hunting,XVII (3):*69-72.
[69] Acatay, A.& Erdem, R. (1946). Due to the convenience that the new additional forest laws will provide in forest protection.*Ankara Journal of the Higher Agricultural Institute5 (20):* 300 - 311.
[70] Acatay, A. (1948). *Hazard key for harmful forest insects.* Ministry of Agriculture, Directorate General of Forestry Publications, Special Issue: 76, IstanbuL. 114 pp.
[71] Acatay, A. (1951). Wald und Weiden. *Försterverband, Ankara*, *pp*. 63 - 66.
[72] Acatay, A. (1951). The Importance of Livestock and Grasslands in Village Development. Yeni Istanbul Newspaper*, 20 February 1951*, p. 2.
[73] Acatay, A (1951). Die Ceder auf dem Bozdağ-Massiv und die orientalische Erle*. Istanbul University,Forestry Faculty Journal, A,1(2)* : 87- 96.
[74] Acatay, A. (1951). Über das Vorkommen von *Betula medwedewi* Rgl. (Det. Rechinger) in der Türkei. *Istanbul University,Forestry Faculty Journal, A, 1 (1):* 212 - 214
[75] Acatay, A. (1952). *Acalla undulana* Wlsghm. als Zederschädling. *J. of İ. U. Faculty of Forestry ,, A, 2 (1)*: 83 - 87.
[76] Acatay, A. (1952). Necessity to improve pastures to reach efficient forest protection. Publications of the Faculty of Forestry University of Istanbul, No.: 21. Third Meeting of the Subcommission for the Mediterranean Countries, İstanbul, pp. 24 - 25.
[77] Acatay, A. (1953). *Waldinsekten und Lebensraum in der Türkei. Grundlagen der Waldentomologie der Türkei.* Veröffentlichungen der Universität Istanbul Nr.: 556, Fakultät für Forstwirtschaft, Nr.: 24, Istanbul, XXIV + 471 S. (übersetzt von Prof. Dr. Erwin Schimitschek).
[78] Acatay, A. (1953). Beitrag zur Biologie des Pinienprozessionsspinners *(Thaumetopoen pityocampa* Schiff. = *Th. wilkinsoni* Tams. *Istanbul University,Forestry Faculty Journal,, A, 3) 1 - 2) :* 29 - 47.
[79] Acatay A (1954) *Kurse in Forstentomologie und Forstschutz*. Hüsnütabiat Matbaası, İstanbul, 44 pp
[80] Acatay, A. (1954). Mistelkraut schädigt unsere Wälder. *J. of İ. U. Faculty of Forestry, B, 4(2)* : 26 - 9.

[81] Acatay, A. (1956). *Pappelkrankheiten und Pappelschutz. "Pappel-Buch".*Istanbul University Publications, Faculty of Forestry No.: 35, İstanbul, pp. 129 - 162.

[82] Acatay,A. (1956). Pyramidenschwarzkiefer *(Pinus nigra* var. *pyramidata). Istanbul University,Forestry Faculty Journal A, 6 (2) :* 92 - 100.

[83] Acatay, A. (1956). Die Preperationen von Larven und Puppen der Tnsektren. *Istanbul University,Forestry Faculty Journal B, 6 (2) :* 19 – 23.

[84] Acatay, A. (1956). Kritik an zwei in Deutschland veröffentlichten Büchern. *Wald und Jagd, Ausgabe 1*: 26-29.

[85] Acatay, A. (1957). History of Forest Entomology and Protection in Turkey. *J. of İ. U. Faculty of Forestry B, VII (2)* : 1 - 3.

[86] Acatay, A. (1957). Beitrag zur Verbreitung von *Pinus silvestris* L. in Anatolien. *IIstanbul University,Forestry Faculty Journal, A, VII (1)* : 114 -117.

[87] Acatay, A. (1958). Ein neuer Phloeosinus aus der Türkei. - 175. Ergänzung zur Morphologie und Systematik der Scolytoidea. *Istanbul University,Forestry Faculty Journal, A, 8 (2) :* 31 - 32. (Übersetzt von Prof. Dr. Karl E. Schedl).

[88] Acatay, A. (1958). Eichörnchenschäden an den Zedernzapfen*., J. of İ. U. Faculty of Forestry A, 8(2*): 1 - 4.

[89] Acatay, A. (1959). *Schutz der Wälder.* Veröffentlichungen der Universität Istanbul Nr.: 824, Fakultät für Forstwirtschaft No. 62, İstanbuL. X +336 pp.

[90] Acatay, A. (1968). Rauchschäden der Kupferhütte in Murgul. *J. of İ. U. Faculty of Forestry A, XVIII (1):* 1 - 17

[91] Acatay, A. (1969). *Practical Forest Entomology.* Istanbul University Publications No.: 1359, Faculty of Forestry No..: 133, İstanbuL. XVI + 182 pp.

[92] Acatay, A. (1969). *Diagnoseschlüssel für schädliche Waldinsekten.* Veröffentlichungen der Universität Istanbul Nr. 1358, Fakultät für Forstwirtschaft, No. 132, İstanbul XII + 154, pp. (3.Auflage).

[93] Acatay, A. (1969). *Chätoptelius vestititus* Muls. als *Pistacia vera* Schädling. *Istanbul University,Forestry Faculty Journal, A, XIX (2)* : 23 - 30.

[94] Acatay, A. & Gülen, İ.(1966). Comparison of German and Austrian State Forest Enterprises in terms of Productivity and Work Volume of Forest Enterprises. *Journal of I.U. Faculty of Forestry, B, XVI (2)* :17 - 25.

[95] Acatay, A (1969) Wie verbrennt man Bodenbedeckung, Trümmer und Shujirat im Wald? *Istanbul University,Forestry Faculty Journal*, B, XIX (2) : 35 - 40.

[96] Acatay, A. (1969). Prof. Dr. E. Schimitschek'in: «Grundzüge der Waldhygiene. *İ Istanbul University,Forestry Faculty Journal, B, XIX (2) :* 100.

[97] Acatay, A. (1970). Lasst uns den Demirköprü-Damm schützen. *Wald and Jagd, März:* 5 - 6.

[98] Acatay, A. (1971). *Behandlung von Baumwunden.* Forstwissenschaftliche Fakultät der Universität Istanbul, I.U. Publication, No.: 1638, O.F. Publication, No.: *162,*İstanbul, vın +39, pp.

[99] Acatay, A. (1971). Über das Auftreten einiger Forstschädlinge in der Türkei.*Anz. für Schädlingskunde und Pflanzenschutz, XLIV (11) :* 161 - 165.

[100] Acatay, A. & Gülen, İ. (1971). Wind damages in Turkish forests. *J. of İ. U. Faculty of Forestry , A, XXI (2)*: 1 - 20.

[101] Acatay, A. (1971). Insektenpräparate in Polyesterharz. *Istanbul University,Forestry Faculty Journal, B, XX (2)* : 60 - 63.

[102] Acatay, A. (1972). *Thaumetopoea Hbst. (*Lep., Thaumetopoeidae ) Arten in der Turkei. XIII. Internationaler Kongress für Entomologie, Moskau, 2 - 9 August,1968, Proceedings, Vol. III: 8 - 9.

[103] Acatay, A. (1972). Auswirkungen der Beweidung auf die Forstprodukte in der Türkei. *Journal of Forestry Research Institute, Ankara,18 (2)* : 3 - 10.

[104] Acatay, A. (1961). Einige Beobachtungen zur Selbstfäule bei Pappeln*., J. of İ. U. Faculty of Forestry B, XI (1)* : 43 - 48.

[105] Acatay, A. (1964). Bedeutung und gesundheitliche Auswirkungen von Medikamenten zur Schädlingsbekämpfung*., J. of İ. U. Faculty of Forestry B, XIV (2):* 1 - 6

[106] Acatay, A. (1967). Naturdenkmäler und Schutz von Vögeln. Veröffentlichungen der Türkischen Naturschutzgesellschaft, Nr.: 10, Seminar über Nationalparks und Naturschutz, pp. 101 - 103.

[107] Acatay, A. (1943). Wie sollten wir die Inselkiefern schützen? Cumhuriyet Zeitung*, 15.5.1943*, p. 2.

[108] Çakırbaş, A. (2020). Nicht-muslimische Abgeordnete in der Großen Nationalversammlung der Türkei (1923-1964). Hiper yayın. Hiperlink Eğitim İlet.Yay., Güngören/ İstanbul.

[109] Acatay, A. (1963). *Praktische Forstentomologie.* Veröffentlichungen der Universität Istanbul Nr. 1068, Fakultät für Forstwirtschaft Nr. *94,*İstanbul XIII + 170, pp.

[110] Acatay, A. (1952). Ein Zedernschädling: *Acalla undulana* WIsghm.Trans. *Ninth Int. Congr.Ent., Vol.1*:708 – 710

[111] Acatay, A. (1959). *Phoracantha semipunctata* Fabr. *(Col. Cerambycidae )in* der Türkei. *Anz. für Schädlingskunde, XXXII (1):* 4 - 5.

[112] Acatay, A. (1959). *Melanophila decastigma* (= *picta* Pall.) und *Gypsonoma(= Semasia) dealbana* Froel. (=. *incarnana* Hw.) in der Türkei. *Anz. für Schädlingskunde, XXXII (5)* : 65 - 68.

[113] Acatay, A (1959). Pappelschädlige in der Türkei. *Anz. für Schädlingskunde, XXXII (9):* 129 - 134.

[114] Acatay, A. (1960). Beitrag zu den Edelkastanienschädlinge in der Türkei. *J. of İ. U. Faculty of Forestry , A, X (1):* 11- 15.

[115] Acatay, A. (1960). Beitrag zu den Forstschädlichen Pilze der Türkei*., J. of İ. U. Faculty of Forestry A, X (2) :* 27 - 36.

[116] Acatay, A. (1960). Eukalyptus schädigt den Tarsus-Karabucak-Wald *Phorocantha semipunctata* Fabr. *J. of İ. U. Faculty of Forestry, B, 10 (1)* : 16 - 20.

[117] Acatay, A. (1963). Die Verbreitung, neue Varietät *(Liquidamber orientalis* var. *suber)* und Insekten von *Liquidamber orientalis* in der Türkei. *Istanbul University,Forestry Faculty Journal, A, XIII (2)* : 40 - 57.

[118] Acatay, A. (1968). Giant Soymuk Beetle (*Dendroctonus micans* Kugel) Georgia (Union of Soviet Socialist Republics) d. *Journal of Forestry Research Institute, Ankara*, 14 (2): 3 - 10. (Übersetzt von Prof. Dr. David Kobachidel).

[119] Acatay, A. (1961). 2 *Cuscuta* - Arten an Pflanzen und Stockausscage der Waldade in der Türkei. *J. of İ. U. Faculty of Forestry A, XI (2)* : 41 - 50.

[120] Acatay, A. (1961f). *Populus euphratica* Oliv. *Istanbul University,Forestry Faculty Journal, B, XI (2)* : 47 - 50.

[121] Acatay, A., Pamay,A & Kalıpsız, A. (1962). Süleymaniye Dişbudak-Forest,thoughts about its Development and Restoration, *Journal of Istanbul University-Faculty of Forestry. B, 12 (2)* ,38 - 54.

[122] Acatay, A. (1963b). Über das Auftreten von einigen Forstschädlingen in der Türkei. *J of applied Entomology, 51 (2) :* 114 - 121.

[123] Acatay, A. (1967 – 1968) *Pinus nigra* var. *pyramidata* und eine interessante Beobachtung. *Mitteilungen der Deutschen Dendrologischen Gesellschaft Nr. 63*:54-55.

[124] Acatay, A. (1961). Waldbrände, Statistik und Sicherheitsmethoden*., J. of İ. U. Faculty of Forestry B, XI (1) :* 35 - 42.

[125] Acatay A (1946) *Forest Protection Guide.* Ministry of Agriculture' General Directorate of Forestry Publications, Istanbul, 65pp.

[126] Acatay, A. (1947). Schwarzziege, der Feind des Waldes. *Wald und Jagd 5:* 94 - 96

[127] Acatay, A.,Gülen İ.& Baş, R.(1978). Haarziege und Waldbeziehungen in der Türkei Wissenschaftlicher und technischer Forschungsrat der Türkei Land- und forstwirtschaftliche Forschungseinrichtung, Projekt Nr: TOAG-100.

**Received:** **21.06.2023**
**Accepted:** **30.08.2023**

**CORRESPONDING AUTHOR**

**Ilhami Kiziroglu**
Ostim Teknik Üniversitesi
Yenimahalle-Ankara-Türkiye

Email:ikiziroglu@gmail.com
ilhami.kiziroglu@ostimteknik.edu.tr

-© by PSP Volume 32– No. 09/2023 pages 2954-2964 Fresenius Environmental Bulletin

# GIS-AHP MULTI CRITERIA NATURE PROTECTION VULNERABILITY EVALUATION METHOD: A CASE STUDY OF TARA NATIONAL PARK, SERBIA

**Darko Lukic[1,*], Marija Peric[2], Slobodanka Stankov[3]**

[1]Military Academy, University of Defence, Pavla Jurisića Šturma 33, 11000 Belgrade, Serbia
[2]Academy of Applied Technical Studies Belgrade, Katarina Ambrozić 3, 11000 Belgrade, Serbia
[3]Western Serbia Academy of Applied Studies, Užice Department, College of Applied Sciences Užice, Trg Svetog Save 34, 31000 Užice, Serbia

## ABSTRACT

The main problem of this research is to determine the "vulnerability" of protected areas on the example of National Park (NP) "Tara". For the purposes of the research, the GIS-AHP Multi Criteria Nature Protection Vulnerability Evaluation Method (GIS-AHP MPVEM) was developed. By applying this method, the obtained results indicate factors that mostly influence on "vulnerability" of the protection regime degree (first, second and third) in the studied area, based on comparisons with the current protection of the area. The spatial protection function of the Zone I is very small (34.7%) and spatial "vulnerability" of the total protection is 41.5%. The result was influenced by the poor relief and communication accessibility of the protected zone. Moderate "vulnerability" (20.8%) is associated with most illegal landfills. Very high (2.5%) and high (0.6%) spatial "vulnerability" refers to the area that is outside of the protection of Zone I or in contact with protection of Zone II. In the future, the obtained parameters should imply new protected zones of NP "Tara", mainly presented in the expansion, i.e. variability of existing boundaries. In accordance with European Union (EU) regulations, recommendations and directives, the Republic of Serbia is obliged to expand the protected areas up to 20% of the total area of its territory.

**KEYWORDS:**
GIS-AHP, spatial "vulnerability", protected zones, National Park "Tara"

## INTRODUCTION

In order to spatial protection, the formation of protected areas of nature is increasingly growing into an activity of global proportions. Introducing the term of nature conservation in the 1980s, scientists have warned to mass extinction of species, especially in biodiversity rich countries [1]. As nature protection is increasingly degraded, humanity is looking for modules on how to highlight and stimulate the protection of natural resources and values [2-3].

The spatial protection in the Republic of Serbia has a long tradition. It is mostly sporadic and unplanned. Natural protected assets cover an area of 6.770 km$^2$ or 7.65% of the total territory of the Republic of Serbia (88.361 km$^2$). It is evident that the surface under protected areas of nature is below the levels of Europe and the world. According to EU directives, the Republic of Serbia is obliged to expand the protected areas up to 20% of the total area of its territory. Consequently, the Spatial Plan of the Republic of Serbia (PPRS) 2021-2035 envisages that the regimes of the first level of protection include 5%, the second level of protection 25% and the third level of protection 67% of the total protected area [4]. The Decree on protection regimes defines general regimes of I (strict protection), II (active protection) and III (proactive protection) degree of spatial protection [5]. According to Maksin, protected areas are generally categorized into three groups of spatial protection (strict protection, selective protection and rehabilitation and renovation) [6].

National parks are the representatives of the most complex spatial protection where strict and selective protection is applied. Of special importance is NP "Tara", whose natural and created values are protected within the most important conservation tool in the EU–NATURA 2000 ecological network [7]. NP "Tara" with "Zaovine" Landscape of outstanding features and Šargan-Mokra Gora Park of Nature will become part of the future cross-border protected area – "Drina" Biosphere Reserve under the UNESCO Man and the Biosphere (MaB) programme. The cross-border position as well as belonging to "Drina-Tara" Euroregion will include the evaluation of protected areas and the establishment of new protection regimes or as Xu proposed in China – the creation of mixed zoning schemes based on the UNESCO MaB programme [8-9].

The main subject, task and goal of this research is to determine the "vulnerability" of the existing

protected area of NP "Tara". Based on the defined criteria that mostly endanger protected zones and lead to spatial "vulnerability" GIS-AHP MPVEM method was created. By applying this method, the obtained research results indicate spatial parts endangered by the action of various factors, based on the comparison with the current state of spatial protection. In the future, the research results should imply a new definition of protection zones and a possible expansion of NP "Tara" in accordance with initiatives, regulations, proposals and laws.

## MATERIALS AND METHODS

In modern environmental research, GIS has been applied alone or in combination with other methods in various aspects of evaluation and zoning of protected areas (Table 1). For the purposes of this research GIS-AHP MPVEM model was developed.

**TABLE 1**
**The applied multi-criteria analysis methods for nature protection zoning.**

| Objective of the evaluation | Method | Reference |
|---|---|---|
| Synthetic evaluation of eco-environment quality | GIS, AHP | [10] |
| Evaluation and protection tool for resources of nature | GIS | [11] |
| Protected area for wildlife sanctuary zoning | GIS, AHP, Fuzzy, MOLA | [12] |
| Environmental impacts in protected areas | GIS | [13] |
| Prevention of unsuitable land use changes and vegetation cover development | AHP, Delphi, MADM, TOPSIS | [14] |
| Conservation of biodiversity | GIS, AHP | [15] |
| Zoning protection of plants for nature conservation | GIS, Fuzzy | [16] |
| Delimitation of landscape | GIS, Fuzzy | [17] |
| Evaluation potential sites for soil and water conservation techniques | GIS, AHP | [18] |
| The suitability of land use | GIS, AHP, Fuzzy logic | [19] |
| Territorial planning of suitable rural areas | GIS, OWA, WLC, AHP | [20] |
| Development of eco-zones in protected areas | GIS, RS, SPCA | [21] |
| Evaluation of sustainable land-use planning | GIS, MCA | [22] |
| Evaluation of the environmental vulnerability | GIS, PPM | [23] |
| Evaluation of ecological habitats vulnerability | GIS, PCA, Fuzzy | [24] |
| Zoning for a multiple-use marine protected area | GIS, AHP, SAW | [25] |
| Assessment of the soil erosion criterion | GIS, AHP, Fuzzy | [26] |
| Environmentally sensitive areas for land use planning | GIS, AHP | [27] |
| Analysis of breeding habitats for recolonizing species | GIS, AHP | [28] |
| Zoning eco-environmental vulnerability for environmental management and protection | GIS, AHP | [29] |
| Assessment of ecosystems vulnerability to fire in managing natural protected areas | GIS, Fuzzy | [30] |
| Zoning ecological red line | GIS, AHP | [31] |
| Nature conservation | GIS, MCDA | [32] |
| Flood hazard zonation | GIS, AHP | [33] |
| Analysis of natural reserves of biodiversity | GIS, AHP, TOPSIS | [34] |
| Ecosystem degradation | GIS, MCA | [35] |
| Population and settlement pressure on protected areas | GIS | [36] |

**TABLE 2**
**Saaty's pair-wise comparison scale for AHP**

| Intensity of relative importance | Definitions |
|---|---|
| 1 | Equal importance |
| 2 | Equal to moderate importance |
| 3 | Moderate importance |
| 4 | Moderate plus |
| 5 | Strong importance |
| 6 | Strong plus |
| 7 | Very strong demonstrated importance |
| 8 | Very, very strong |
| 9 | Extreme importance |

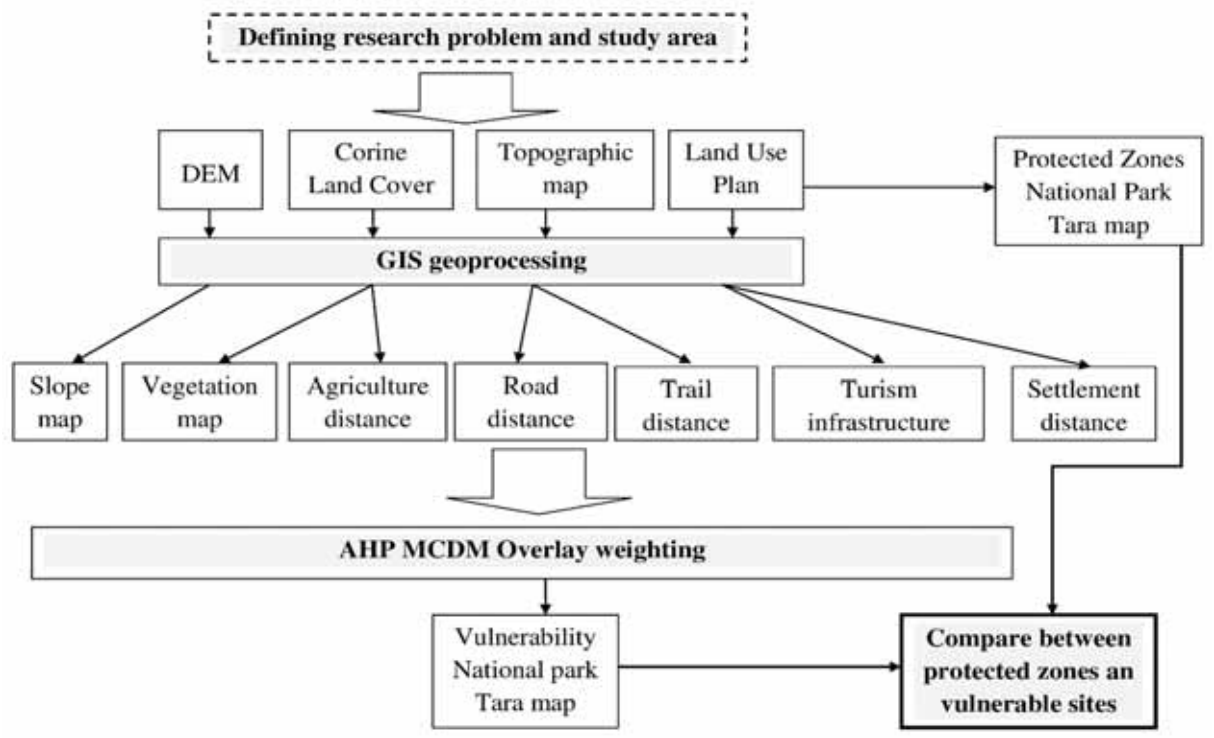



**FIGURE 1**
**Steps of GIS-AHP MPVEM model**

$$r_{ix,iy} = \sum_{i=1}^{M} W_i V_{i;ix,iy} ix = 1, ix = 1, \dots, nx, iy = 1,2, \dots, ny \quad (2)$$

The proposed model consists of several logically related steps presented in (Figure 1). The multicriteria analysis model is based on integration of widely applicable AHP and Weighted Overlay tool. Based on a large number of factors that may have an impact on the endangermentanalysis of existing protected zones in NP "Tara", AHP integrated in GIS can provide appropriate manipulation and presentation of data with consistent evaluation.

AHP is procedure for modeling, based on raster data in a multi criteria hierarchical configuration [37]. The original Saaty´stechnique [38] involves a nine-level of assessment (Table 2).

Based on the opinion of one or more experts, the procedure involves assessing the importance of each individual factor. Then, the nine-level assessment system is applied to a two-dimensional n×n reciprocal AHP matrix, where each criterion is compared with each of their in order to get relative weights expressed in numerical scale values as it follows$w = (w_1, w_2, \dots, w_n)$. Normalization of the sum of rows is perform by dividing the sum of each row by the number of rows. The result of the calculation is a priority vector, i.e. a vector of the eigenvalues of the matrix. In the standard AHP, the eigenvector method (EV) is used to determine the weights of the criteria, that is, the main right vector of the eigenvalues of the matrix A [31]. This vector is obtained by solving a linear system (1):

$$Aw = \lambda w \quad (1)$$

where λ is the largest eigenvalue of the matrix A, and w is required vector of weight criterion.

Based on the results of the previous step (1), each criterion received an appropriate weight coefficient, defining its relative value in relation to the desired outcome [32]. The final convenience map is obtained by calculating the total benefit of each raster cell using equation 2:

where $r_{ix,iy}$ is the cell value $ix$, $iy$ on the final convenience map and $W_i$ is the weight coefficient.

The consistency of the matrix is controlled by consistency parameters $CI$ (Consistency Index), $RI$ (Random Index), $CR$ (Consistency Ratio), $\lambda_{max}$ (Eigenvalue – sum of the products between each element of the Eigen vector and column totals) and n (Number of factor), using equation 3:

$$CR = \frac{CI}{RI}; CI = \frac{\lambda_{max} - n}{n-1} \quad (3)$$

If $n$ is assumed to be the number of conditional factors, then the total number of comparisons that one expert should make is $\frac{n(n-1)}{2}$ [35]. Consequently, this procedure is adequate only in cases where no more than ten factors are involved (Table 3), but in some cases, such as our work, a minor degree of inconsistency is fully acceptable [39].

**TABLE 3**
**Average Random Consistency Index with consistency scale rate**

| Scale rate | High Consistency | | | | | Average Consistency | | | | | Low Consistency | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix size | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| (RI) | 0 | 0 | 0.58 | 0.9 | 1.12 | 1.24 | 1.32 | 1.41 | 1.45 | 1.49 | 1.51 | 1.54 | 1.56 | 1.57 | 1.59 |

The proposed model is consistent if $CR < 0.1$. It can be interpreted that the assessment matrix is inconsistent with less than 10%, or it is consistent with over 90%. In the following procedure, it is recommended the sharpness of the model to be tested.

**Study area.** The first step was to define the research area. Than, the problem of the research was identified too. Based on geographical characteristics, possible ways of solving the observed problem were also defined. NP "Tara" is located in the western part of the Republic of Serbia, in the bend of the Drina River, on the border with Bosnia and Herzegovina.With an area of 24.991,82 ha, it covers the largest part of Tara Mountain, as a significant resource of the Western SerbiaTourist Region.The problem of the paper refers to the "vulnerability" of protected zones in NP "Tara". In accordance with the problem, the criteria that mostly endanger protected zones and lead to their "vulnerability" have been defined.

**Criteria modeling and standardization.** Modeling and standardization of the necessary criteria for the spatial analysis was performed in the next step. In the previous practice of solving the problem of spatial protection evaluation different spatial criteria have been applied (Table 4).

Topographic criteria have an impact on the spatial accessibility. Higher altitudes and slopes prevent access to human activity. Areas with greater percentage of slope are less frequented than others [34]. Altitude has a significant impact on processes that can affect environmental vulnerability [29]. Lower altitudes and smooth topography are accessible for human activity. Higher altitudes are less accessible and endangered [16].

The quality of vegetation diversity is an important criterion in spatial protection [15]. An area with large number of endemic species has a higher value in spatial protection. So far 1013 plant species have been identified on Tara Mountain. Picea omorika, the relict and endemic species, indicates the importance and potential of Tara Mountain as floristic and vegetation diversity of Serbia and Balkan Peninsula [71]. In vegetation criterion the highest value is assigned to coniferous forests, mixed forests and meadows [43].

Representation of animal species is interdependent with vegetation, water resources and topographic criteria [12]. Areas with a large number of registered wildlife habitats have the greatest value in spatial protection [44]. Over 53 species of mammals, 153 species of birds and 37 species of fish have been registered on Tara Mountain.

.

**TABLE 4**
**The most frequently used criteria for nature protection in previous research**

| Criteria | Reference | Relevant research |
|---|---|---|
| Topography | [40], [41], [16], [21], [20], [42], [27], [43], [29],[34], [44] | yes |
| Vegetation diversity | [45], [11], [46], [12], [15], [16], [47], [20], [21], [48], [43], [27], [29], [49], [34] | yes |
| Wildlife distribution | [45], [12], [50], [16], [48], [27], [44] | no |
| Water bodies | [12], [15], [16], [47], [23], [27], [42], [29], [51] | no |
| Hydro meteorology | [52], [42], [43], [29], [44] | no |
| Road distance | [53], [12], [15], [16], [47], [21], [20], [48], [43], [54], [29], [34] | yes |
| Settlement and infrastructure pressure | [53],[45], [55], [56], [57], [13], [58], [15], [47], [21], [20], [59], [43],[ 54], [34], [60], [61], [62], [36] | yes |
| Agriculture sites | [45], [12], [63], [47], [19], [20], [23], [48], [54], [42], [29], [34] | yes |
| Cultural presence and tourism sites | [12], [64], [65], [20], [27], [66], [67], [68], [60] | yes |
| Natural risk | [45], [23], [27], [42], [47], [33] | no |
| Pollution risk | [55], [27], [34] | no |
| Natural protected area | [69], [70], [15], [47], [67], [34] | yes |

Roads increase human pressure on natural areas [12]. The most endangered areas are in the immediate vicinity of the roads. The quality of roads does not have the same value when it comes to the accessibility of spatial protected areas [16]. The distance of more than 3000 m from the roads does not negatively affect the biodiversity preservation [15]. For that reason, roads are evaluated differently depending on their importance, traffic frequency etc

Settlements and different types of infrastructure have a negative impact on spatial protection. Primarily it refers to hydropower plants, tourist infrastructure, rural and weekend settlements. Protected sites close to these objects are at a higher risk of disturbance by the local residents [53, 16, 34].

Agricultural areas disrupt biodiversity through human activity and through the risk of soil and groundwater pollution [34]. Natural spatial areas at a greater distance from agricultural areas have a greater protective value. Therefore, areas near crops have a greater tendency to be damaged than others [34].

After defining the criteria, it is necessary to assign credit values. On the basis of assigned credit values on a five-point scale, an evaluation of the spatial endangerment will be performed according to the appropriate spatial values (Table 5).

In the last step, the appropriate cartographic material was selected. Then, selected material was adapted to the appropriate digital form suitable for the application of GIS-AHP MPVEM (Table 6).

**Model evaluation processing.** In the further evaluation step, ArcGIS Desktop 10.6 software was used. The first step in evaluating alternatives in AHP method is normalization (Table 7).

Criteria weights were automatically computed in decision support tool using AHP Excel Template Update Version 2018-09-15, where criterion maps were obtained through appropriate standardization (Figure 2a).

**TABLE 5**
**Criteria for assessing the endangerment to protected areas of Tara National Park**

| | Criteria | Assigned score | | | | |
|---|---|---|---|---|---|---|
| | | Very Low | Low | Moderate | High | Very High |
| C1 | Slope (°) | 40< | 30-40 | 20-30 | 10-20 | <10 |
| C2 | Vegetation type | Transitional woodland/shrub | Broad-leaved forest | Mixed forest | Coniferous forest | Natural grasslands |
| C3 | Distance to large roads (m) | 1000< | 750-1000 | 500-750 | 250-500 | <250 |
| C4 | Distance to trails (m) | 200< | 150-200 | 100-150 | 50-100 | <50 |
| C5 | Distance to vilages and settlment (m) | 2000< | 1500-2000 | 1000-1500 | 500-1000 | <500 |
| C6 | Distance to tourism infrastructure (m) | 3000< | 2000-3000 | 1000-2000 | 500-1000 | <500 |
| C7 | Distance to agriculture sites (m) | 2000< | 1500-2000 | 1000-1500 | 500-1000 | 500< |

**TABLE 6**
**Materials and used methods of criteria map preparation**

| Criteria | Data material | Scale | Geoprocessing method | Reference |
|---|---|---|---|---|
| Slope | Digital Terrain Model (DTM) | 25 x 25m | Reclassify, slope surface analyses | [72] |
| Roads distance<br>Trails distance | Digital topographic map 50 (TK50) | 1: 50000 | Radial distance, Reclassify | [73] |
| Settlement and villages distance<br>Tourism infrastructure distance | Spatial Plan of the special purpose area of the National Park “Tara” (Reference map 3 and 4 Natural resources, environmental protection, natural and cultural values, tourism, implementation of the plan) | 1: 50000 | Radial distance, Reclassify | [74] |
| Protected zone distance | | 1: 50000 | Clip, Reclassify | |
| Vegetation<br>Agriculture sites | Corine Land Cover 2018 | 25 x 25m | Radial distance, Reclassify | [75] |

**TABLE 7**
**Materials Criteria Comparison Matrix**

| Criteria | | C1 | C2 | C3 | C4 | C5 | C6 | C7 | Weight |
|---|---|---|---|---|---|---|---|---|---|
| Slope | C1 | 1 | 8 | 3 | 5 | 5 | 5 | 5 | 0.4200 |
| Vegetation | C2 | 1/8 | 1 | 1/5 | 1/3 | 1/7 | 1/8 | 1/5 | 0.0245 |
| Roads distance | C3 | 1/3 | 5 | 1 | 3 | 1 | 1 | 3 | 0.1529 |
| Trails distance | C4 | 1/5 | 3 | 1/3 | 1 | 1/2 | 1/3 | 1 | 0.0632 |
| Vilages and settlment | C5 | 1/5 | 7 | 1 | 2 | 1 | 1 | 1 | 0.1189 |
| Tourism infrastructure | C6 | 1/5 | 8 | 1 | 3 | 1 | 1 | 1 | 0.1308 |
| Agriculture sites | C7 | 1/5 | 5 | 1/3 | 1 | 1 | 1 | 1 | 0.0898 |

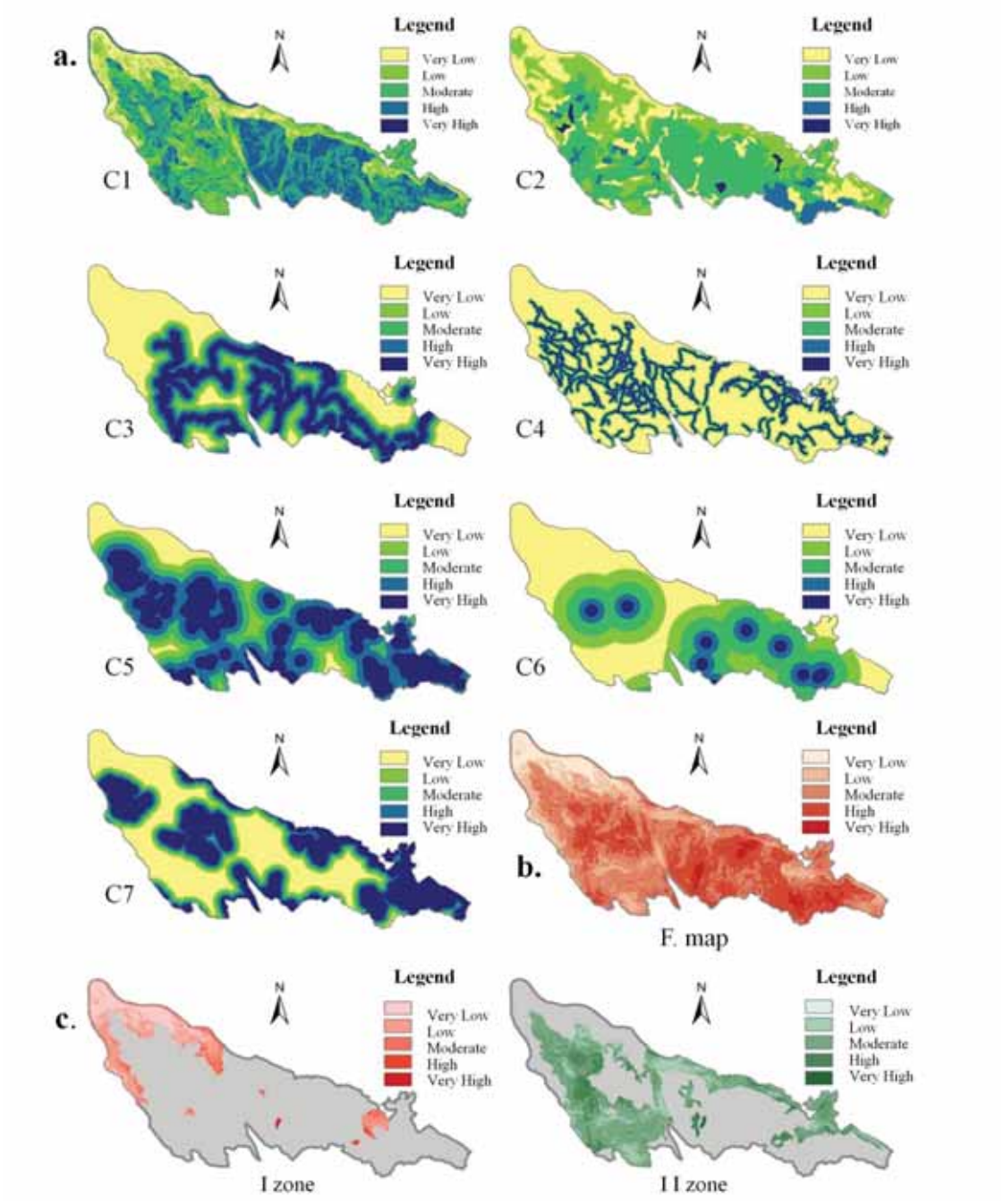



**FIGURE 2**
**Map of standardized criteria (a). Final spatial vulnerability map (b). Map of spatial vulnerability of protected zones (c)**

**TABLE 8**
**Endangerment of Tara National Park**

| Area | Very Low | Low | Moderate | High | Very High |
|---|---|---|---|---|---|
| Zone I (km²) | 11.6 | 13.9 | 6.9 | 0.8 | 0.2 |
| Zone II (km²) | 0.1 | 13.2 | 48.6 | 17.0 | 0.6 |
| Total (km²) | 11.7 | 27.1 | 55.6 | 17.9 | 0.8 |

As the obtained $CR$ is 0.05 it can be claim that the proposed model has a satisfactory consistence. As said before the proposed model is consistent if $CR < 0.1$. In the further procedure, the criterion maps were integrated into the final map based on the obtained weight vectors using the ArcGIS Weighted Overlay tool (Figure 2b). The last step is the final evaluation of endangered area protection obtained model with the existing model of protected areas defined in the PPRS 2021-2035 [74]. This step was performed by comparing the potentially endangered area with the actual protected area of NP "Tara" (Figure 2c).

## RESULTS AND DISCUSSION

In the last step, using the GIS-AHP MPVEM, the protected zones were compared with the obtained spatial "vulnerability" map. The obtained results are shown in Table 8.

Based on an existing document PPRS 2021-2035 [74], the function of spatial protection of Zone I is very small and amounts 34.7%, while the spatial "vulnerability" is 41.5% of the total protection. The result was influenced by the poor relief and communication accessibility of the protected zone confirmed by the results of previous research in this area [36]. A small part of the vulnerable area within the protected Zone I is topographically accessible and is located near the roads in a moderately vulnerable area of 20.8%. According to Jordá-Borell et al., [76] smooth topography is associated with most illegal landfills. Very high (2.5%) and high (0.6%) spatial "vulnerability" refers to segments that are outside the protection of Zone I or are in contact with protection of Zone II (Figure 2c).

When it comes to the protected area of Zone II the results of the evaluation showed a significantly higher degree of "vulnerability". Very high (0.1%), high (21.4%) and medium (61.4%) "vulnerability" of the mentioned protected area were observed. The small (21.4%) and very small percentage (0.7%) of protected zones "vulnerabilities" were influenced by similar factors as in Zone I. The most important factor that influenced the greater "vulnerability" is the presence of the local population, in our case defined as population. The presence of the population has a high impact on the degradation of the protected area[35]. Economic activity has a significant role in spatial endangering and the most important factor that has been noticed is the unplanned, i.e. illegal construction of private weekend facilities. According to Caniani et al., [24] every human activity affects the reduction of the number of plant and animal habitats. Another important factor of "vulnerability" that was observed is the presence of infrastructure intended for mass tourism within or in the immediate vicinity of protected areas. Ristić et al., [13] noticed similar factors of spatial endangering that arose as a consequence of mass tourism development and good communication connections in the area of NP "Kopaonik".

Negative aspects of endangering the protected area of NP "Tara" can be eliminated or partially mitigated by taking appropriate activities, laws and measures. The first one is to redefine the existing protected area and adopt regulatory acts in favor of expanding protected Zone I. In that context, the current cross-border and European initiatives are very important. These initiatives indicate and dictate that in coming period more than 20% territory of the Republic of Serbia should be protected. This will not be possible without the implementation, i.e. the application of the concept of sustainable development in tourism, where one of the solutions is the successful development of ecological tourism. Ecotourism is just one of the solutions where it is necessary to reconcile the negative aspects of mass tourism and economic activities with nature protection [77]. As one of the most influential factors on the spatial "vulnerability" was the communication availability, it is necessary to limit access to vehicles as well as the number of visitors in certain parts of the protected zones of NP "Tara".

## CONCLUSION

The presented method is conceived by AHP multi criteria analysis and shows the natural and social factors that affect the protection of NP "Tara". The method is characterized by compactness and is suitable for adapting and evaluating the "vulnerability" of other categorized protected areas (natural monument, protected habitat, landscape of exceptional features and park of nature). This would require some modification and selection of criteria for assessing "vulnerability" in line within the natural and created features of the new evaluated area. In addition to the good sides, the proposed method has certain weaknesses and shortcomings. In order to maintain a high level of consistency, the number of criteria has been reduced. Cultural heritage sites that create pressure on the environment

based on tourist visits and endangered the protected area were not included. Also, the sensitivity analysis was not applied in the paper. Elaboration of different scenarios through sensitivity analysis would increase the percentage of subjectivity in the assessment of protected area valuation as presented by Comino et al., [47]. In this context, the basic idea of presented GIS-AHP MPVEM is a solution for quick comparison of the desired and actual state of the protected area. Thus, model methodological possibilities have not been exhausted, but it is necessary to further develop them.

## REFERENCES


[1] Otero, I., Boada, M. and Tabara, J.D. (2013) Social-ecological heritage and the conservation of Mediterranean landscapes under global change. A case study in Olzinelles (Catalonia). Land Use Policy. 30(1), 25-37.

[2] Admiraal, J.F., Musters, C.J.M. and De Snoo, G.R. (2016) The loss of biodiversity conservation in EU research programmes: Thematic shifts in biodiversity wording in the environment themes of EU research programmes FP7 and Horizon 2020. Journal for Nature Conservation. 30, 12-18.

[3] Zinngrebe, Y. (2016) Learning from local knowledge in Peru – Ideas for more effective biodiversity conservation. Journal for Nature Conservation. 32, 10-21.

[4] Institute of Architecture and Urbanism of Serbia (IAUS) (2021) Spatial plan of the Republic of Serbia 2021-2035 – Draft plan. [In Serbian]. Available online: https://www.mgsi.gov.rs/sites/default/files/PPRS%20Nacrt.pdf (31 January 2020).

[5] Official Gazette of the Republic of Serbia (2012) Regulation on protection regimes, 31 [In Serbian]. Available online: https://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/vlada/uredba/2012/31/1 (31 January 2020).

[6] Maksin, M. (2013) Tourism and space. Belgrade: Singidunum University, Department of Tourism and Hotel Management [In Serbian]. 1-338.

[7] Orlikowska, E.H., Roberge, J.M., Blicharska, M. And Mikusiński, G. (2016) Gaps in ecological research on the world's largest internationally coordinated network of protected areas: A review of Natura 2000. Biological Conservation. 200, 216-227.

[8] Turnock, D. (2002) Ecoregion-based conservation in the Carpathians and the land-use implications. Land Use Policy. 19, 47-63.

[9] Xu, W., Li, X., Pimm, S.L., Hull, V., Zhang, J., Zhang, L., Xiao, Y., Zheng, H. and Ouyang, Z. (2016) The effectiveness of the zoning of China's protected areas. Biological Conservation. 204, 231-236.

[10] Ying, X., Guang-Ming, Z., Gui-Qui, C., Lin, T., Ke-Lin, W. and Dao-You, H. (2007) Combining AHP with GIS in synthetic evaluation of eco-environment quality – A case study of Hunan Province, China. Ecological Modelling. 209, 97-109.

[11] Radović, D. (2008) Evolving GIS at Tara National Park (Serbia). Bulletin of the Serbian Geographical Society. 88(3), 87-102.

[12] Hajehforooshnia, S., Soffianian, A., Salman Mahiny, A. and Fakheran, S. (2011) Multi objective land allocation (MOLA) for zoning Ghamishloo Wildlife Sanctuary in Iran. Journal for Nature Conservation. 19, 254-262.

[13] Ristić, R., Marković, A., Radić, B., Nikić, Z., Vasiljević, N., Živković, N. and Dragićević, S. (2011) Environmental impacts in Serbian ski resorts. Carpathian Journal of Earth and Environmental Sciences. 6(2), 125-134.

[14] Sadegh Ravesh, M.H., Zentabian, G., Ahmadi, H. and Khosravi, H. (2012) Using analytic hierarchy process method and ordering technique to assess de-desertification alternativies. Case study: Khezrabad, Yazd, Iran. Carpathian Journal of Earth and Environmental Sciences. **7**(3), 51-60.

[15] Bottero, M., Comino, E., Duriavig, M., Ferretti, V. and Pomarico, S. (2013) The application of a Multi criterion Spatial Decision Support System (MCSDSS) for the assessment of biodiversity conservation in the Province of Varese (Italy). Land Use Policy. 30(1), 730-738.

[16] Zhang, Z., Sherman, R., Yang, Z., Wu, R., Wang, W., Yin, M., Yang, G. and Ou, X. (2013) Integrating a participatory process with a GIS-based multi-criteria decision analysis for protected area zoning in China. Journal for Nature Conservation. 21(4), 225-240.

[17] Bata, T., Mezósi, G. and Meyer, B.C. (2014) Landscape units for Hungary using multiresolution segmentation of GEO-DATA and Fuzzy analysis. Carpathian Journal of Earth and Environmental Sciences. 9(2), 45-56.

[18] Krois, J. and Schuite, A. (2014) GIS-based multi-criteria evaluation to identify potential sites for soil and water conservation techniques in the Ronquillo watershed, northern Peru. Applied Geography. 51, 131-142.

[19] Houshhyar, E., Sheikh Davoodi, M.J., Almassi, M., Bahrami, H., Azadi, H., Omidi, M., Sayyad, G. and Witlox, F. (2014) Silage corn production in conventional and conservation tillage systems. Part I: Sustainability Analysis Using Combination of GIS/AHP and Multi-Fuzzy Modeling. 39, 102-114.

[20] Romano, G., Dal Sasso, P., Trisorio Liuzzi, G. and Gentile, F. (2015) Multi-criteria decision analysis for land suitability mapping in a rural area of Southern Italy. Land Use Policy. 48, 131-143.
[21] Nandy, S., Singh, C., Das, K.K., Kingma, N.C. and Kushwaha, S.P.S. (2015) Environmental vulnerability assessment of eco-development zone of Great Himalayan National Park, Himachal Pradesh, India. Ecological Indicators. 57, 182-195.
[22] Nguyen, T.T., Verdoodt, A., Tran, V.Y., Delbecque, N., Tran, T.C. and Ranst, E.V. (2015) Design of a GIS and multi-criteria based land evaluation procedure for sustainable land-use planning at the regional level. Agriculture, Ecosystems and Environment. 200, 2-11.
[23] Shao, H., Sun., X., Tao, S., Xiang, Z. and Xian, W. (2015) Environmental vulnerability assessment in middle-upper reaches of Dadu River watershed using projection Pursuit model and GIS. Carpathian Journal of Earth and Environmental Sciences. 10(4), 133-146.
[24] Caniani, D., Labella, A., Lioi, D.S., Mancini, I.M. and Masi, S. (2016) Habitat ecological integrity and environmental impact assessment of anthropic activities: A GIS-based fuzzy logic model for sites of high biodiversity conservation interest. Ecological Indicators. 67, 238-249.
[25] Habtemariam, B.T. and Fang, Q. (2016) Zoning for a multiple-use marine protected area using spatial multi-criteria analysis: The case of the Sheik Said Marine National Park in Eritrea. Marine Policy. 63, 135-143.
[26] Haouses, C., Dridi, H., Saint Gerand, T. and Kalla, M. (2016) Integrating GIS and AHP method for erosion vulnerability determination for Libido watershed (Eastern Algeria). Carpathian Journal of Earth and Environmental Sciences. 11(1), 5-16.
[27] Leman, N., Firuz Ramli, M. and Khirotdin, R.P.K. (2016) GIS-based integrated evaluation of environmentally sensitive areas (ESAs) for land use planning in Langkawi, Malaysia. Ecological Indicators. 61 (2), 293-308.
[28] Macmillan, H., Moore, A.B., Augé, A.A. and Chilvers, B.L.(2016) GIS-based multi-criteria analysis of breeding habitat as for recolonizing species: New Zealand sea ilions. Ocean & Costal Management. 130, 162-171.
[29] Nguyen, A.K., Liouc, Y.A., Li, M.H. and Tran, T.A. (2016) Zoning eco-environmental vulnerability for environmental management and protection. Ecological Indicators. 69, 100-117.
[30] Semeraro, T., Mastroleo, G., Aretano, R., Facchinetti, G., Zurilini, G. and Petrosillo, I. (2016) GIS Fuzzy Expert System for the assessment of ecosystems vulnerability to fire managing Mediterranean natural protected areas. Journal of Environmental Management. 168, 94-103.
[31] Chunye, W. and Delu, P. (2017) Zoning of Hangzhou Bay ecological red line using GIS-based multi-criteria decision analysis. Ocean& Costal Management. 139, 42-50.
[32] Esmail, B.A. and Geneletti, D. (2017) Multi-criteria decision analysis for nature conservation: A review of 20 years of applications. Methods in Ecology and Evolution. 9, 42-53.
[33] Khaleghi, S. and Mahmoodi, M. (2017) Assessment of flood hazard zonation in a mountainous area based on GIS and Analytical Hierarchy Process. Carpathian Journal of Earth and Environmental Sciences. 12(1), 311-322.
[34] Sánchez-Lozano, J.M. and Bernal-Conesa, J.A. (2017) Environmental management of Natura 2000 network areas through the combination of Geographic Information Systems (GIS) with Multi-Criteria Decision Making (MCDM) methods. Case study in south-eastern Spain. Land Use Policy. 63, 86-97.
[35] Ciocănea, C.M., Corpade, P.C., Onose, D.A., Vănău, G.O., Maloş, C., Petrovici, M., Gheorghe, C., Dedu, S., Manta, N. and Szép, R.E. (2019) The Assessment of lotic ecosystems degradation using Multi-criteria analysis and GIS techniques. Carpathian Journal of Earth and Environmental Sciences. 14(2), 255-268.
[36] Telbisz, T., Brankov, J. and Ćalić, J. (2020) Topographic and lithologic controls behind mountain depopulation in Zlatibor District (Western Serbia). Journal of Mountain Science. 17(2), 271-288.
[37] Malczewski, J. (2006) GIS based multi criteria decision analysis: a survey of the literature. International Journal of Geographical Information Science. 20(7), 703-726.
[38] Saaty, T.L. (2012) Decision Making for Leaders: The Analytic Hierarchy Process for Decisions in a Complex World. Third Revised Edition. Pittsburgh: RWS Publications. 83-97.
[39] Mu, E. and Pereyra-Rojas, M. (2017) Understanding the Analytic Hierarchy Process. In Practical Decision Making. An Introduction to the Analytic Hierarchy Process (AHP) Using Super Decisions V2. Springer: Berlin, Germany. 2, 7-22.
[40] Condorachi, D. (2011) Geomorphological mapping using GIS for large tableland areas – An example for Fălciu hills, in Eastern Romania. Carpathian Journal of Earth and Environmental Sciences. 6(2), 115-124.

[41] Ćalić, J., Gaudenyi, T., Milošević, M.V., Štrbac, D. and Milivojević, M. (2012) Geomorpho-metrical method for delineation of plains – Case study of the South-Eastern (Serbian) segment of the Pannonian Plain. Carpathian Journal of Earth and Environmental Sciences. 7 (2), 239-248.

[42] Jusys, T. (2016) Quantifying avoided deforestation in Pará: Protected areas, buffer zones and edge effects. Journal for Nature Conservation. 33, 10-17.

[43] Franco-Maass, S., Burrola-Aguilar, C., Arana-Gabriel, Y. and García-Almaraz, L.A. (2016) A local knowledge-based approach to predict anthropic harvesting pressure zones of wildedible mushrooms as a tool for forest conservation in Central Mexico. Forest Policy and Economics. 73, 239-250.

[44] Koch, R., Almeida-Cortez, J.S. and Klein-schmit, B. (2017) Revealing areas of high nature conservation importance in a seasonally dry tropical forest in Brazil: Combination of modelled plant diversity hot spots and threat patterns. Journal for Nature Conservation. 35, 24-39.

[45] Phua, M.H. and Minowa, M. (2005) A GIS-based multi-criteria decision making approach to forest conservation planning at a landscape scale: a case study in the Kinabalu Area, Sabah, Malaysia. Landscape and Urban Planning. 71, 207-222.

[46] Cvjetićanin, R. and Novaković, M. (2010) Floristic diversity of beech, fir and spruce forest (piceo-fago-abietetumčolić 1965) in the "Tara" National Park. Bulletin of the Faculty of Forestry. 102, 129-142.

[47] Comino, E., Bottero, M., Pomarico, S. and Rosso, M. (2014) Exploring the environmental value of ecosystem services for a river basin through a spatial multi criteria analysis. Land Use Policy. 36, 381-395.

[48] Caniani, D., Labella, A., Lioi, D.S., Mancini, I.M. and Masi, S. (2016) Habitat ecological integrity and environmental impact assessment of anthropic activities: A GIS-based fuzzy logic model for sites of high biodiversity conservation interest. Ecological Indicators. 67, 238-249.

[49] Finsinger, W., Morales-Morino, C., Gałka, M., Valsecchi, V., Bojović, S. and Tinner, W. (2012) Holocene vegetation and fire dynamics at Crveni Potok, a small mire in Dinaric Alps (Tara National Park, Serbia). Quaternary Science Reviews. 167, 63-77.

[50] Rotich, D. (2012) Concept of zoning management in protected Areas. Journal of Environment and Earth Science. 2(10), 173-183.

[51] Borek, L. (2018) Eutrophication risk of water in the Manor-park channels: Different ways of evaluation. Carpathian Journal of Earth and Environmental Sciences. 13(2), 409-421.

[52] Ranković, N., Poduška, Z., Nedeljković, J. and Nonić, D. (2015) Influence of temperature and rainfalls on the number of visitors in the National Park "Tara". Bulletin of the Faculty of Forestry. 112, 97-102.

[53] Geneletti, D. (2004) A GIS-based decision support system to identify nature conservation priorities in an alpine valley. Land Use Policy.21(2), 149-160.

[54] Hervé, M., Albert, C.H. and Bondeau, A. (2016) On the importance of taking into account agricultural practices when defining conser-vation priorities for regional planning. Journal for Nature Conservation. 33, 76-84.

[55] Guiqin, W., Li, Q., Guoxue, L. and Lijun, C. (2009) Landfill site selection using spatial information technologies and AHP: A case study in Beijing, China. Journal of Environmental Management. 90, 2414-2421.

[56] Tomićević, J., Shannon, M.A. and Milovanović, M. (2010) Socio-economic impacts on the attitudes towards conservation of natural resources: Case study from Serbia. Forest Policy and Economics. 12, 157-162.

[57] Ladányi, Z., Rakonczai, J. and Deák, Á.J. (2011) A Hungarian landscape under strong natural and human impact in the last century. Carpathian Journal of Earth and Environmental Sciences. 6(2), 35-44.

[58] Blagojević, I. (2012) Sustainable Landscape Management in Tara National Park (Village Jagoštica, Serbia). Geographica Pannonica. 16(3), 94-102.

[59] Đorđević, D.S., Šećerov, V., Filipović, D. and Lukić, B. (2016) The impact of infrastructure planning in spatial plans on carrying capacity assessment in mountain tourist areas. Spatium. 35, 79-86.

[60] Brankov, J., Jojić Glavonjić, T., Milovanović Pešić, A., Petrović, M.D. and Tretiakova, T.N. (2019) Residents'perceptions of tourism impact on community in National Parks in Serbia. European Countryside. 11(1), 124-142.

[61] Kroh, P., Struś, P., Wrońska-Wałach, D. and Gorczyca, E. (2019) Map of landslides on the commune scale based on spatial data from airborne laser scanning. Carpathian Journal of Earth and Environmental Sciences. 14(1), 155-164.

[62] Zorić, M., Đukić, I., Kljajić, L.J., Karaklić, D. and Orlović, S. (2019) The Possibilities for Improvement of Ecosystem Services in Tara National Park. Poplar. 203, 53-63.

[63] Benyhe, B. and Kiss, T. (2012) Morphometric analysis of agricultural landforms in lowland ploughed-fildes using high resolution digital elevation model. Carpathian Journal of Earth and Environmental Sciences. 7(3), 71-78.
[64] Ilieş, A., Dehoorne, O. and Ilieş, D.C. (2012) The Cross-border territorial system in Romanian-Ukrainian Carpathian area. Elements, mechanisms and structures generating premises for an integrating cross-border territorial system with tourist function. Carpathian Journal of Earth and Environmental Sciences. 7(1), 27-38.
[65] Tomićević, J., Bjedov, I., Gudurić, I., Obratov-Petković, D. and Shannon, M.A. (2012) Tara National Park – Resources, Management and Tourist Perception. In Barbara Sladonja (Ed.), Protected Area Management. 73- 92.
[66] Špulerova, J., Hrnčiarova, T., Piscova, V., Vlachovičova, M., Kalivoda, H., Kanka, R., Dobrovodska, M., Kenderessy, P., Miklósova, V., Drábova, M. and Belčákova, I. (2016) Sustainable tourism development in a selected area of the Low Tatras National Park – landscape planning versus urban planning. Carpathian Journal of Earth and Environmental Sciences. 11(2), 485-496.
[67] Brankov, J., Petrović, M.D., Radovanović, M., Tretiakova, T.N. and Syromiatnikova, Y.A. (2017) Geography and Environment – Analysis of indicators of sustainable development of tourism. International Journal of Cognitive Research in Science, Engineering and Education (IJCRSEE). 5(1), 131-140.
[68] Đorđević, N., Lakićević, M. and Milićević, S. (2018) Tourism destinations benchmarking analysis of the Tara and Kopaonik National Parks. Economics – Theory and Practice. 11(3), 52-70.
[69] Gobbi, M., Riservato, E., Bragalanti, N. and Lencioni, V. (2012) An expert-based approach to invertebrate conservation: Identification of priority areas in central-eastern Alps. Journal for Nature Conservation. 20, 274-279.
[70] Klaučo, M., Weis, K., Stankov, U., Arsenovic, D. and Marković, V. (2012) Ecological significance of land-cover based on interpretation of human-tourism impact. A case from two different protected areas (Slovakia and Serbia). Carpathian Journal of Earth and Environmental Sciences.7 (3), 231-246.
[71] Radović, D., Stevanović, V., Marković, D., Jovanović, S., Džukić, G. and Radović, I. (2005) Implementation of GIS technologies in assessment and protection of natural values of Tara national park. Archives of Biological Sciences. 57(3), 193-204.
[72] Military Geographical Institute [MGI] (2003) Digital Terrain Model (DTM) Available online: URL:http://www.vgi.mod.gov.rs/lat. (Accessed date:20. 04. 2022).
[73] Military Geographical Institute [MGI] (2006) Digital topographic map 50 (TK50) URL: http://www.vgi.mod.gov.rs/lat. (Accessed date: 15. 05 .2022).
[74] Yugoslav Institute of Urbanism and Housing [JUGINUS] (2019) Spatial plan of the special purpose area of the "Tara" National Park – Draft plan, [In Serbian]. Available online: https://www.mgsi.gov.rs/sites/default/files/PPPPN%20NP%20TARA%20-%20tekst.pdf. (31 January 2020).
[75] European Environment Agency [EEA] (2021) Cover (CLC) 2018, Version 2020_20u1. Available online: https://land.copernicus.eu/pan-european/corine-land-cover/clc2018 (31 January 2020).
[76] Jordá-Borrell, R., Ruiz-Rodríguez, F. and Lucendo-Monedero, A.L. (2014) Factor analysis and geographic information system for determining probability areas of presence of illegal landfills. 37, 151-160.
[77] Gigović, L.J., Pamučar, D., Lukić, D. and Markovic, S. (2016) GIS-Fuzzy DEMATEL MCDA model for the evaluation of the sites for ecotourism development: A case study of "Dunavski ključ" region, Serbia. Land Use Policy. 58, 348-365.

**Received:** **21.06.2023**
**Accepted:** **30.08.2023**

**CORRESPONDING AUTHOR**

**Darko Lukic**
Military Academy
University of Defence
Belgrade 11000 – Serbia

e-mail: hadzidarko.lukic@gmail.com

-© by PSP Volume 32– No. 09/2023 pages 2965-2972 Fresenius Environmental Bulletin

# THE ASSESSMENT OF ECOLOGICAL RISK AND THE POLLUTION OF HEAVY METALS IN AGRICULTURAL SOILS NEAR AN INDUSTRIAL AREA ELHADJAR - ANNABA - ALGERIA

**Daroui Atika[1,2,*], Guilane Sarra[3], Guerroudj Fatima[4], Tahar Wafa[5], Eddine Ahmed[6], Benslama Mohamed[2]**

[1]Faculty of Natural and Life Sciences, Department of Plant Biology and Ecology, Ferhat Abbes University, Sétif 19000, Algeria.
[2]Laboratory of Soil and Sustainable Development, Badji Mokhta - Annaba University, P.O. Box 12, Annaba 23000, Algeria
[3]Laboratory of Environmental Engineering, Process Engineering Department, Faculty of Technology, Badji Mokhta - Annaba University, P.O. Box 12, Annaba 23000, Algeria
[4]Laboratory of Arthropod Biodiversity and Ecology, Constanitine 25000, Algeria
[4] Laboratory of Plant Biology and Environment, Annaba 23000, Algeria
[5]Laboratory of water conservatory management soil and forest, Faculty of Nature and Life Sciences, University of Tlemcen, Tlemcen 13000, Algeria

## ABSTRACT

The aim of the study was to assess the risk of soil contamination, as well as the accumulation and distribution patterns of heavy metals in each soil profile in Annaba, northeastern Algeria. The study also aimed to calculate the ecological risk index and determine the degree of pollution in agricultural soils.The results showed that the soil pH was slightly neutral to alkaline, and the metal concentrations were within safe limits. The four indices used in the study, namely (Cf), (Er), (Igeo), and (RI), indicated that the mean Er values for the four metals were all under 40. This suggests a relatively low danger level for these metals in the soil, indicating that the soil in the study area is not severely contaminated with toxic metals. However, a correlation analysis revealed that some heavy metals had a significant relationship with pH, indicating that the neutral to alkaline pH levels in the agricultural soils may have contributed to the mobility of heavy metals and their availability for uptake by crops. Overall, the study provides valuable information for policymakers and researchers interested in assessing the potential risks associated with heavy metal accumulation in agricultural soils in Annaba, Algeria. The study's findings suggest that ongoing monitoring of soil quality and the potential impact of heavy metal accumulation to protect soil quality.

**KEYWORDS:**
Contamination, heavy metals, ecological risk, agricultural soils

## INTRODUCTION

Soils have been identified as major storage sites for heavy metals that are released into the environment. Their concentrations have increased due to human activities, particularly from the use of pesticides and fertilizers [1], as well as from the chemical, mining, and steel industries [2]. Most heavy metals do not undergo microbial or chemical degradation, as they are non-degradable, and their total concentrations remain in the environment for extended periods [3, 4]. Among the heavy metals, elements like Zn and Cu are involved in biochemical reactions and are therefore essential to living organisms, although they can become toxic depending on their concentrations [5]. On the other hand, metals like Cd and Pb are major soil contaminants and have no known function in organisms, representing high toxicity for plants and animals even at low concentrations [6]. Moreover, the accumulation of heavy metals in agricultural soil results in a reduction of crop productivity or even a complete loss of yield [7].

The direct effects of heavy metals on plants include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress, while the indirect effects could involve a reduction in beneficial microorganisms in the soil due to heavy metals toxicity, which reduces the enzyme activities necessary for plant development. The availability of metals in the soil depends on physical, chemical, or biological processes [8]. The main factors that govern the mobility of an element in the soil are pH, redox potential, organic matter content, texture, mineral composition, and the chemical form of the element [7]. Understanding the mobility and bioavailability of these elements in the soil is essential for assessing risks [9] and developing a plan for remediation, including Mercury (Hg), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb), Arsenic (As), Chromium (Cr), and Zinc (Zn) [10]. Heavy metals have been classified as priority control contaminants by the United States Environmental Protection Agency

(USEPA), and they have attracted increasing attention throughout many regions of the world due to their potentially hazardous, chronic, and irreversible features [11, 12]. Although Cu is a necessary trace element, excessive amounts can harm health [13]. Anthropogenic sources, including industrial pollution from non-ferrous mining and smelting operations, are the major causes of heavy metal contamination [14]. Various analyses have been used to evaluate the contamination intensity of heavy metals in agricultural soils, such as pollution index, enrichment factor, and ecological risk index, although it is difficult to determine the most appropriate method for evaluating such cases. Furthermore, multivariate and geostatistical analyses are commonly performed [14].

The problem addressed in this study is the impact of heavy metal pollution on agricultural lands located near the iron industry. Several methods are used to investigate and understand the processes and sources of various heavy metals generated and accumulated in agricultural soils. In this study, the objectives were twofold: (i) to assess the accumulation and distribution patterns of heavy metals in each soil profile, and (ii) to calculate the ecological risk index, a diagnostic tool for determining the degree of pollution in agricultural soils. By achieving these objectives, the study aims to provide a better understanding of heavy metal contamination in the study area, which can help in developing appropriate remediation measures.

## MATERIALS AND METHODS

**Description of the study area.** The study region, "Annaba," is located in northeastern Algeria, as shown in Figure 1. This area is known for its Mediterranean climate, which is characterized by mild, wet winters and hot, dry summers. The topography of the area is generally flat, with the western edge rising up to the foothills of the Edough Mountains. The soil in this region is mostly made up of recent alluvium with a clayey texture.

**Sample Collection.** Soil sampling was carried out in two campaigns: the first campaign occurred during the summer season, at the beginning of June, while the second campaign was conducted after the winter season. During the first sampling campaign, we created soil profiles, which were identified by GPS. The same sites were sampled during the second campaign (Figure 1). The soil profiles were 1.50 m wide and varied in depth according to site conditions, with a depth of 140 cm for all profiles.

The soil samples were taken from the top 0-30 cm arable layer, as well as from subsoil 1 (30-80 cm) and subsoil 2 (80-140 cm), air-dried, sieved to 2 mm, and stored at room temperature until analysis.

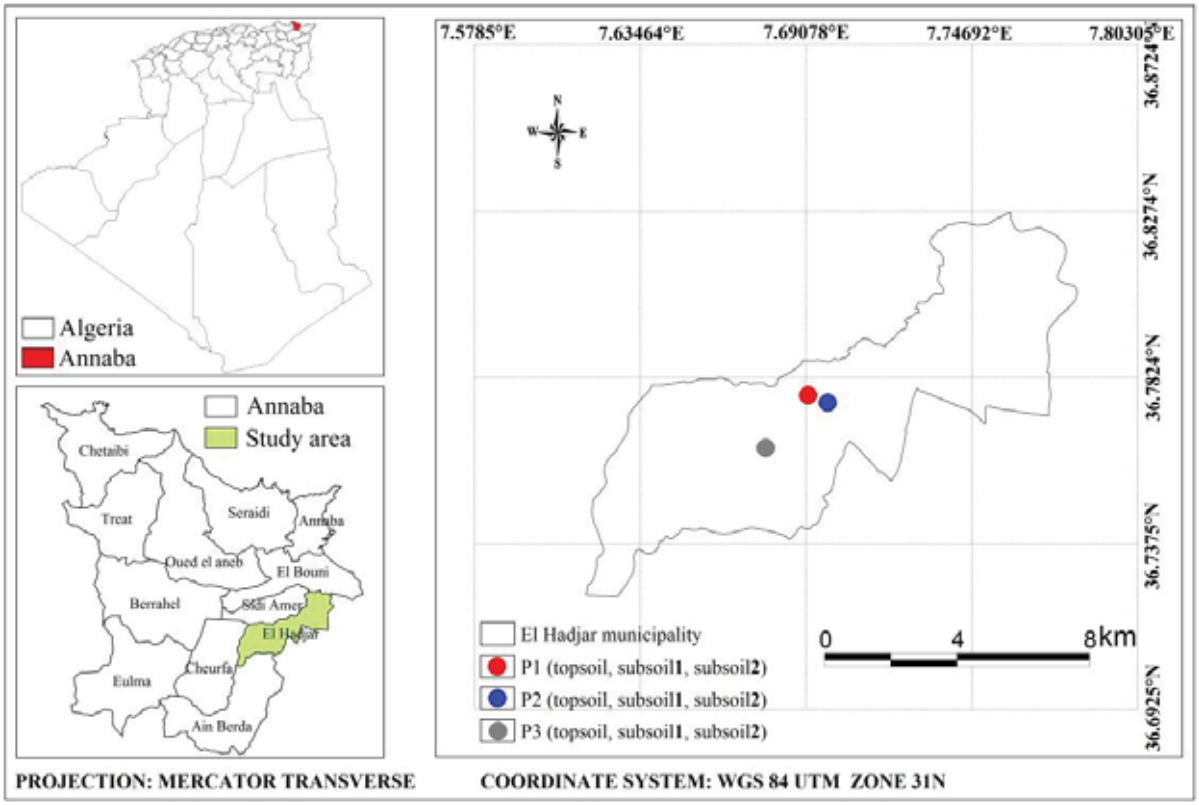



**FIGURE 1**
**Location of the study and sampling sites on Annaba in Algeria.**

**Sample analysis.** All samples were analyzed to quantify the concentrations of heavy metals (Cr, Zn, Cu, Ni, Pb). The sodium nitrate extraction (NaNO3-Ex method) was used to measure these concentrations, and appropriate dilutions were made before electro-thermal atomic absorption spectrometry with background compensation was used for determination.

**Assessment of Soil Contamination Risk. Contamination factor ($C_f$).** The contamination factor (Cf) is a method used to evaluate the level of certain hazardous metals in soil. Many previous studies have used the Cf as a straightforward and useful approach for identifying contamination by toxic metals. The formula for calculating the contamination factor (Cf) is as follows (1):

$$C_f = \frac{\text{C Metal}}{\text{C Backgraound}} \quad (1)$$

Where C metal is the detected metal concentration in the soil sample and C background represents the background reference concentration values for the individual metals [15]. The several categories into which the contamination factor ($C_f$) is classified are shown in Table 1.

**Geoaccumulation Index.** Initially defined the geoaccumulation index (Igeo), which has been widely applied in trace metal investigations of sediments and soils [16]. Igeo was calculated in accordance with [16] is provided in Eq 2. The data were interpreted using the Igeo classifications listed in Table 1.

$$\text{Igeo} = \log_2\left(\frac{Cn}{1.5\ Bn}\right) \quad (2)$$

Where, Cn is the measured concentration of the studied metal n in the soil, and Bn is the geochemical background concentration of the metal n of average shale.

**Ecological risk factor (Er).** The ecological risk factor (Er) is an empirical method for evaluating the ecological risk in soil based on metal toxicity and environmental response factors. The Er was calculated utilizing Equation (3) as stated in the study by [17] Table 1 details the categorization of soil pollution based on Er.

$$E_r = T_r \times C_f \quad (3)$$

Where Cf is the contamination factor, which was discussed in the previous section, and Tr is the toxic response factor values for each particular metal, which are given in Table 2.

**TABLE 1**
**Contamination indices classification for soil.**

| Index | Description | References |
|---|---|---|
| Contamination factor (Cf) | Cf<1 LOW Contamination<br>1≤cf<2 LOW To Moderate Contamination<br>2≤cf<3 Moderate Contamination<br>3≤cf<4 Moderate to High Contamination<br>4≤cf<5 High Contamination<br>5≤cf<6 High Contamination to very High Contamination<br>Cf≥6 Very High Contamination | [17,18] |
| Geoaccumulation index (Igeo) | Igeo>5 very strongly polluted<br>4≤Igeo≤5 strongly polluted to very strongly polluted<br>3≤Igeo≤4 strongly polluted<br>2≤Igeo≤3 moderatly to strongly polluted<br>1≤Igeo≤2 moderatly polluted<br>0≤Igeo≤1 unpolluted to medratly polluted<br>Igeo=0 unpolluted | [19,20] |
| Ecological Risk Factor (Er) | Er < 40 Low risk<br>40 ≤Er < 80 Moderate risk<br>80 ≤Er < 160 Considerable risk<br>160 ≤Er < 320 High risk<br>Er ≥ 320 Very high risk | [21,22] |
| Potential Ecological Risk Index (RI) | RI < 150 Low risk<br>150 ≤RI < 300 Moderate risk<br>300 ≤RI < 600 Considerable risk<br>RI ≥ 600 High Risk | [23,24] |

**TABLE 2**
**Toxic-response factor values of toxic metals [17, 25]**

| Metals | Cr | Pb | Ni | Cu | Zn |
|---|---|---|---|---|---|
| Toxic Response Factor | 2 | 1 | 5 | 2 | 1 |

**TABLE 3**
**Descriptive Statistics for Heavy Metal in Tested Agricultural Soil Samples (mg/kg), SGV Soil Guideline Values of heavy metals in agricultural soil samples.**

| | Mean | SD | Sum | Min | Max | SGV |
|---|---|---|---|---|---|---|
| pHeau | 7,65 | 0,55 | 160,68 | 6,54 | 7,63 | - |
| Cu | 5,02 | 0,94 | 105,41 | 3,69 | 4,93 | 63 |
| Zn | 44,72 | 14,76 | 939,21 | 29,82 | 43,38 | 200 |
| Cr | 24,87 | 3,96 | 522,26 | 17,97 | 24,61 | 64 |
| Pb | 25,26 | 19,60 | 530,56 | 11,78 | 22,23 | 70 |
| Ni | 16,17 | 2,58 | 339,47 | 9,8 | 16,97 | 50 |

**Potential Ecological Risk Index (RI).** The potential ecological risk index (RI) is a technique for evaluating dangers that soil provides to the environment. It is a thorough evaluation of a contaminated site to determine the potential ecological danger. The following Equation was used to determine the RI [19].

$$RI = \Sigma Er \quad (4)$$

Where Er denotes the ecological risk factor for each soil sample component's hazardous metal element. Table 1 illustrates the amounts of hazardous metals according to their potential ecological impact.

**Statistical analysis.** Statistical methods were used to analyze the effects of two experimental factors: depth (with three levels: topsoil, subsoil 1, and subsoil 2) and sampling campaign (summer season and winter season). Basic statistical parameters such as minimum, maximum, mean, standard deviation (SD), and standard error (SE) were calculated, along with correlation analysis, to evaluate the data in terms of its distribution and correlation among the studied parameters. To determine whether there were any differences in the concentrations of heavy metals (Cr, Ni, Cu, Pb, and Zn) and to validate the results, a two-way analysis of variance (ANOVA) was conducted using Origin Statistics Version 18. Significant differences were considered to exist at $p < 0.05$.

## RESULTS AND DISCUSSION

**Concentration of heavy metals in the soil samples.** In this study, the pH of the agricultural soils was found to be slightly neutral to alkaline, with an average value of 7.87. Both soil pH and sampling campaign were relatively homogeneous. The means of the metal concentrations in the collected soil samples were observed and are detailed in Table 1, along with a description of the heavy metal concentrations at different sampling sites. Figure 2 also shows the metal concentrations in the collected soil samples.

It should be noted that the descriptive statistics in Table 1 provide valuable information on the distribution of heavy metal concentrations in the study area. The mean, standard deviation, minimum, maximum, and standard error values for each heavy metal can help in understanding the extent of variability and possible sources of contamination. Furthermore, the background or threshold values (soil quality guideline values 'SGV') for heavy metals provided are crucial in assessing the potential environmental impact of the heavy metal concentrations found in the soil samples.

Overall, the results of the study suggest that the concentrations of heavy metals in the study area are within safe limits and do not pose a significant threat to the environment or human health. The correlation analysis in Table 2 shows that some heavy metals have a significant relationship with each other, indicating that they may share common sources or pathways of contamination. The negative correlation between pH and heavy metals indicates that pH may play a role in controlling the mobility and availability of heavy metals in the soil.

A correlation matrix was used to investigate potential relationships between heavy metals in soil samples. Typically, a correlation coefficient close to 1.0 suggests a strong relationship between the tested metals in such statistical analyses. However, a correlation coefficient of 1.0 is only reached when a variable is related to itself. Table 2 shows the correlation matrix between pH and the five heavy metals. Significant correlation coefficients with values (Table 1) were observed at a significance level of 0.05. Significant correlations were found for the relationships between Cu and Zn, Cu and Cr, Zn and Cr, Cu and Ni, Zn and Ni, Cr and Ni, Cu and Pb, and Pb and Zn. Additionally, negative correlations were observed between pH and all heavy metals in the correlation matrix. Overall, the correlation matrix helped to identify that several pairs of heavy metals share common natural sources due to the geological structure of soil and rocks in the study area.

The high correlation observed between Cr, Cu, Zn, Ni, and Pb in the soils of the study area suggests that these heavy metals are primarily derived from natural weathering of parent materials or the crust in the study area [26]. Conversely, low or no correlations between heavy metals reflect anthropogenic sources of these metals. The high degree of correlation and significant relationship between metals indicates their identical behavior during transport in the coastal environment [27].

**TABLE 4**
**Pearson correlation matrix for the heavy metals; pH and CE in agricultural soil samples.**

| Annaba | pHeau | Cu | Zn | Cr | Pb | Ni |
|---|---|---|---|---|---|---|
| pHeau | 1,00 | | | | | |
| Cu | -0,24 | 1,00 | | | | |
| Zn | -0,33 | **0,84*** | 1,00 | | | |
| Cr | -0,07 | **0,45*** | **0,64*** | 1,00 | | |
| Pb | -0,28 | **0,69*** | **0,68*** | 0,03 | 1,00 | |
| Ni | 0,08 | **0,53*** | 0,30 | **0,61*** | 0,11 | 1,00 |

***= correlation is significant at the 0,05 level**

The strong positive relationships observed for all metals could be due to the similarity in their sources, both natural and anthropogenic. Previous studies by [28, 29] found that Cu and Zn were positively correlated, suggesting a common origin of these metals in their respective study areas. [30] also suggested that this could be due to similar uptake and release mechanisms by organisms living in or overlying the soils.

Overall, the results indicate that there may be some level of heavy metal contamination in the study area, likely from both natural and anthropogenic sources. This highlights the importance of regular monitoring and management of the coastal environment to prevent further contamination and ensure its sustainability for agricultural practices and human health.

**Distribution of heavy metals in the soil samples.** In this study, ANOVA was used to test if there were significant differences in the concentrations of heavy metals between the two sampling campaigns, and also to check if there were any significant differences in the depth of the soil samples.

The results of the ANOVA showed that all tested heavy metals were not significantly different between the two sampling campaigns, meaning that there were no significant changes in the concentrations of heavy metals in the soil over time. However, it is worth noting that there was a slight decrease in the concentrations of heavy metals in the second sampling campaign compared to the first. This could be due to several factors such as changes in environmental conditions, or possibly a reduction in anthropogenic activities in the study area.

Overall, the concentrations of heavy metals found in the soil samples were generally low in relation to the norms values, indicating that the soil in the study area is relatively free from heavy metal contamination.

The impact of pH on the mobility and availability of heavy metals in soil is well-known in environmental research. In this study, the relationship between pH and heavy metal concentration was investigated in agricultural soil samples. The statistical analysis revealed that the pH levels were not significantly different from each other, with a p-value of greater than 0.05. Moreover, the neutral pH value was found to enhance the mobility of heavy metals in the soil samples [31].

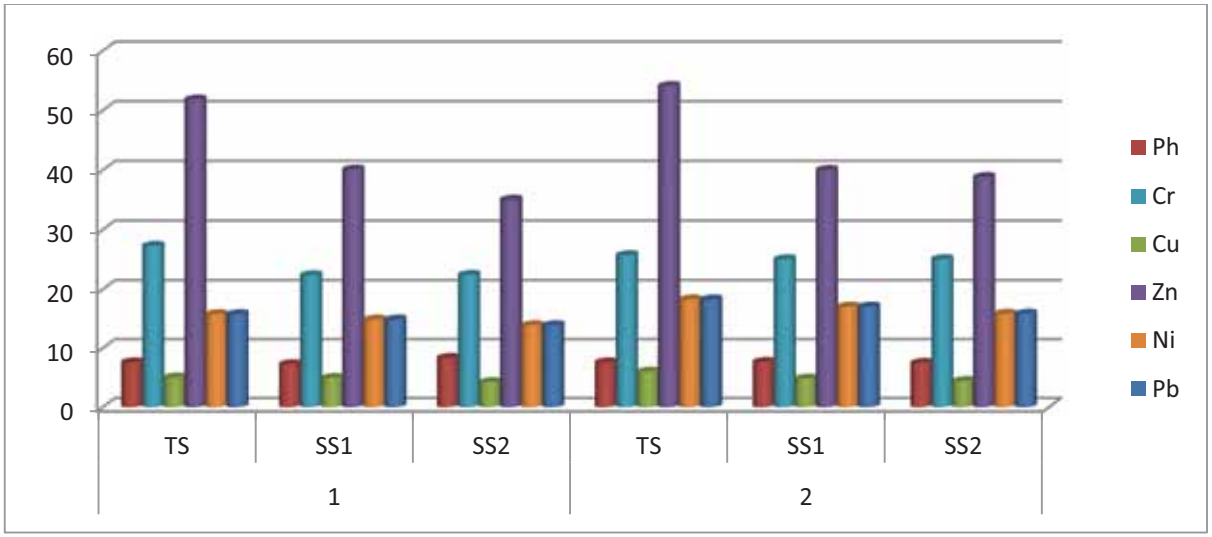



**FIGURE 2**
**Concentrations of heavy metals (mg/Kg) in soils samples (TS: top soil, SS1: sub soil1, SS2: Sub soil 2), (1: summer season, 2= winter season)**

The pH values of the soil samples collected for this study were generally neutral to alkaline in the subsoil layer. This could be attributed to the excessive use of organic fertilizers and continuous cultivation of crops, which might have increased the pH values of the agricultural soils over time. This observation is consistent with the findings of a previous study by [32], who reported that the use of organic fertilizers led to an increase in soil pH values. It is important to note that the neutral to alkaline pH values in the agricultural soils may have contributed to the mobility of heavy metals and their availability for uptake by crops, potentially posing a risk to human health and the environment.

The distribution of heavy metals across different soil horizons was analyzed, and the results showed that the highest concentration of heavy metals was found in the topsoil (TS) layer. The concentration of heavy metals decreased in the subsurface layer SS1 and then increased again in the SS2 layer. The heavy metals were ranked in the order of their concentrations in the soil horizons as follows: Zn > Pb > Cr > Ni > Cu. It is worth noting that although heavy metal concentrations were high in the soil horizons, they were still within the acceptable limits of soil quality guideline values (SQV).

Among all the heavy metals analyzed, zinc was found to have the highest concentration in all soil horizons. This is likely due to the application of zinc fertilizers in agricultural fields, which has been previously reported to lead to the accumulation of zinc in soil [33]. Nonetheless, it is important to note that the concentrations of zinc and other heavy metals were within acceptable limits, indicating that the agricultural soils in the study area are not significantly contaminated with heavy metals.

Most of the agricultural soils studied had nickel concentrations below the background norms for agricultural soil. However, one soil sample showed lower concentrations of chromium, copper, and lead, possibly due to waste dumping by chemical industries or the use of inorganic fertilizers and sewage in agricultural soils. The second soil sample showed a reduction in heavy metal concentrations, which could be due to factors such as high solubility at low pH. However, there was an exception in the second soil sample in SS2, where the alkaline pH is believed to have reduced the solubility of heavy metals in agricultural soil. Overall, the results suggest that heavy metal contamination in agricultural soils in the study area is relatively low and does not pose a significant risk to the environment or human health.

**Assessment of Contamination and Environmental Risk.** The study assessed the presence of toxic metals in surface soils using four indices: Contamination factor (Cf), Index of geoaccumulation (Igeo), Ecological risk factor (Er), and Potential Ecological Risk Index (RI). These indices were chosen because they are based on the soil's hazardous metal background reference value, which helps to demonstrate the level of external contamination.

The results of the assessment showed that the five studied metals (Cr, Cu, Pb, Zn, and Ni) had low contamination levels (Cf < 1). The mean Er values for the metals were Cu (0.10012), Zn (0.25921), Ni (0.36158), Cr (0.53876), and Pb (1.64178), indicating a relatively low danger level for these metals in the soil. All four metals had mean Er values under 40, indicating that the soil in the study area is not severely contaminated with toxic metals.

**TABLE 5**
**presents the results of the evaluation using these four indices. The table provides information on the contamination factor (Cf), Index of geoaccumulation Ecological risk factor (Er), and Potential Ecological Risk Index (RI) for each of the five studied metals (Cr, Cu, Pb, Zn, and Ni).**

| | | Mean | SD | Sum | Min | Max |
|---|---|---|---|---|---|---|
| Cu | **Cf** | 0,02 | 0,00 | 0,38 | 0,01 | 0,03 |
| | **I geo** | 9,68 | 0,26 | 183,94 | 9,26 | 10,34 |
| | **Eri** | 0,10 | 0,02 | 1,90 | 0,07 | 0,16 |
| Zn | **Cf** | 0,26 | 0,08 | 4,93 | 0,18 | 0,56 |
| | **I geo** | 12,16 | 0,34 | 230,98 | 11,68 | 13,30 |
| | **Eri** | 0,26 | 0,08 | 4,93 | 0,18 | 0,56 |
| Cr | **Cf** | 0,27 | 0,03 | 5,12 | 0,20 | 0,31 |
| | **I geo** | 10,50 | 0,16 | 199,47 | 10,07 | 10,72 |
| | **Eri** | 0,54 | 0,06 | 10,24 | 0,40 | 0,62 |
| Pb | **Cf** | 0,33 | 0,25 | 6,24 | 0,20 | 1,37 |
| | **I geo** | 10,29 | 0,56 | 195,47 | 9,74 | 12,52 |
| | **Eri** | 1,64 | 1,27 | 31,19 | 1,00 | 6,86 |
| Ni | **Cf** | 0,07 | 0,01 | 1,37 | 0,04 | 0,09 |
| | **I geo** | 11,23 | 0,26 | 213,42 | 10,52 | 11,54 |
| | **Eri** | 0,36 | 0,06 | 6,87 | 0,22 | 0,44 |
| | **RI** | 2,90 | 1,39 | 55,13 | 1,88 | 8,52 |

The Igeo values were used to explain the soil quality. The Igeo values of Cr, Cu, Pb, Zn, and Ni indicated no pollution in the examined soil samples. This means that the soil quality in the study area is relatively good and not contaminated by the studied metals.

The Potential Ecological Risk Index (RI) is a measure of the distinct biological ecosystems' sensitivity to toxic contaminants. The study found that the study area has a low ecological risk level (RI <150), indicating that the presence of the studied metals in the soil is not likely to pose a significant ecological risk.

## CONCLUSION

Based on the study results, it can be concluded that agricultural soils in Annaba, northeastern Algeria, have relatively low levels of heavy metal pollution, with metal concentrations within safe limits. However, the correlation analysis revealed that certain heavy metals had significant relationships with each other and with pH, suggesting a potential risk to human health and the environment. The neutral to alkaline pH levels in the agricultural soils may have contributed to the mobility of heavy metals and their availability for uptake by crops. Overall, the study provides valuable information on the concentrations and distribution of heavy metals in agricultural soils in Annaba. These findings can serve as a baseline for future studies and can help in developing effective strategies for soil management practices and sustainable land use in the region.

## REFERENCES

[1] Sands, M. (2003) The Bioaccumulation of Heavy Metals in Fishes: A Review. Journal of the Marine Biological Association of the United Kingdom. 83(4), 725-725.

[2] Wong, M.H. (2012) Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere. 87(10), 1089-1097

[3] Lepp, N.W. (2012) A review of the biogeochemistry and ecotoxicology of selenium and its impacts on the environment. Environmental Toxicology and Chemistry. 31(11), 2462-2473.

[4] Athar, M. and Vohora, S.B. (2001) Heavy metal toxicity: A review of sources and effects in the environment. Pakistan Journal of Pharma-ceutical Sciences. 14(2), 1-22.

[5] T. Alharbi, A.S. El-Sorogy, S. Qaysi, F. Alshehri (2021), Evaluation of groundwater quality in central Saudi Arabia using hydrogeochemical characteristics and pollution indices Environ. Sci. Pollut. Res. 10.1007/s11356-021-14575-1

[6] Xu, Y., Zhao, Y., Sun, L., Zhao, X., Wang, L., Sun, X. and Liu, C. (2018) Heavy metal contamination of agricultural soils affected by mining activities around the Ganxi River in Chenzhou, Southern China. Environmental Science and Pollution Research. 25(30), 30334-30346.

[7] Szolnoki, Z. and Farsang, A. (2013) Heavy metal concentrations in game animals from a lead mining area (Southern Hungary). Environmental Science and Pollution Research. 20(2), 1002-1012.

[8] Souza, R.M. (2015) The occurrence, fate and effects of pharmaceuticals and personal care products (PPCPs) in the environment. Environmental Science and Pollution Research. 22(21), 16650-16671.

[9] Mattos, B.D., Garcia, G.V., Lucheta, F. and de Oliveira, L.M. (2016) Occurrence and fate of endocrine disrupting compounds (EDCs) in wastewater treatment plants and their environmental impact. Environmental Science and Pollution Research. 23(4), 3614-3638.

[10] Chibuike, G.U. and Li, Y. (2019) Heavy metal content of poultry feeds and the accumulation of metals in poultry manure and tissues. Environmental Monitoring and Assessment. 191(9), 548

[11] Giller, K.E. and Mc, C. (1988) Nitrogen Fixation by Legumes in Tropical and Subtropical Agriculture. Advances in Agronomy. 41, 223-264.

[12] Rodrigues, A.R., do Nascimento, C.W., and da Silva, M.G. (2013) Phytoremediation of arsenic-contaminated waters using aquatic macrophytes: A review. International Journal of Phytoremediation. 15(2), 193-207.

[13] Zhou, L., Li, Y., Wang, Y., Dong, Q., Yang, X. and Li, X. (2018) Health risk assessment of heavy metal exposure to street dust in the zinc smelting district, Northeast China. Science of the Total Environment. 610-611, 1089-1096.

[14] López, J.A. (2012) Environmental impact of mining and mineral processing: Management, monitoring, and auditing strategies. CRC Press. 73.

[15] Turekian, K.K. (1962) The oceans. In Encyclopedia of Earth Sciences. Springer. 9, 422-425.

[16] Muller, G. (1969) Index of geo-accumulation in sediments of the Rhine River. GeoJournal. 2(3), 108-118.

[17] Hakanson, L. (1980) An ecological risk index for aquatic pollution control. A sedimentological approach. Water Research. 14(8), 975-1001.
[18] Wang, J., Cui, B., Yang, Z., Chen, L. and Guo, L. (2018) The response of macroinvertebrates to environmental gradients and anthropogenic stressors in the upper Han River basin, China. Science of the Total Environment. 615, 1020-1029.
[19] Zahra, N.A. (2014) Heavy metal contamination of soils and vegetables in suburban areas of Lahore, Pakistan. Journal of Chemistry. 2014, 1-8.
[20] Bintal, A., Ahmad, S., Tariq, S. and Khan, M.A. (2009) Investigation of heavy metal pollution in soil and vegetables grown in the vicinity of combined industrial estate, Karachi. Pakistan Journal of Botany. 41(1), 41-50.
[21] Rostami, R., Karimi, N. and Vaezi, A.R. (2021) Risk assessment of heavy metals in soil, water and plant samples of Ghareh-Sou catchment, Iran. Environmental Monitoring and Assessment. 193(5), 1-15.
[22] Mahabadi, H.A., Yousefi, N., Naddafi, K., Yaghmaeian, K. and Ghaffari, H.R. (2020) Health risk assessment of heavy metals in atmospheric particulate matter in Tehran, Iran. Environmental Monitoring and Assessment. 192(11), 702.
[23] Kang, Y., Wang, X., Wang, H., Ma, Y., Xu, B. and Li, B. (2020) Heavy metal pollution in urban soils with various types of land use in a rapidly developing city of China. Environmental Pollution. 267, 115609.
[24] Hu, X., Zhang, Y., Ding, Z., Wang, T., Lian, H., Sun, Y. and Wu, J. (2013) Health risks associated with heavy metals in fine particulate matter: a case study in a heavily polluted city in northern China. Environmental Research. 121, 1-8.
[25] Xu, L., Zhang, Y., He, M. and Zhang, G. (2008) Assessment of heavy metal pollution and human health risk in urban soils of steel industrial city (Anshan), Liaoning, Northeast China. Ecotoxicology and Environmental Safety. 71(2), 221-228.
[26] Cai, L., Xu, Z., Ren, Z. and Zhao, Y. (2015) Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. Environmental Earth Sciences. 73(1), 197-205.
[27] Alagarsamy, R. (2006) Heavy metal concentrations in the surface sediments of the intertidal region of the Gulf of Mannar, Southeast Coast of India. Journal of Environmental Biology. 27(4), 657-661
[28] Ramessur, R.T. (2004) Heavy metal concentrations in sediments of Grand Port lagoon, Mauritius. Environment International. 30(8), 1049-1058.
[29] Yap, C.K., Ismail, A., Tan, S.G. and Omar, H. (2005) Heavy metal (Cd, Pb, Zn, Cu, Cr, Fe and Mn) concentrations in the green-lipped mussel Perna viridis (Linnaeus) collected from some wild and aquacultural sites in the West Coast of Peninsular Malaysia. Food Chemistry. 90(2), 207-214.
[30] Broman, D., Hansson, S., Jansson, M. and Andersson, A. (1991) The significance of metal-organic complexation on the bioavailability of cadmium and copper in natural waters. Water Research. 25(4), 461-468.
[31] Kabata-Pendias, A. (2011) Trace Elements in Soils and Plants. CRC Press. 52.
[32] Shamim, S., Ali, S., Ullah, S., Muhammad, S., Rizwan, M., Khan, M.R. and Hameed, M.T. (2019) Accumulation of heavy metals in soils, plants, and hair samples: an assessment of heavy metal pollution in the vicinity of Tannery Industrial Zone, district Kasur, Pakistan. Environmental Science and Pollution Research. 26(1), 873-885.
[33] Garg, V.K., Yadav, P., Mor, S., Singh, I. and Kumar, A. (2014) Heavy metals in vegetables collected from production and market sites of a peri-urban area of Delhi, India. Food and Chemical Toxicology. 70, 48-55.

**Received:** **29.07.2023**
**Accepted:** **12.ß8.2ß23**

**CORRESPONDING AUTHOR**

**Daroui Atika**
Laboratory of Soil and Sustainable Development
Badji Mokhta - Annaba University
Annaba 23000 – Algeria

e-mail: darouichaima@gmail.com

-© by PSP Volume 32– No. 09/2023 pages 2973-2980 Fresenius Environmental Bulletin

# SEASONAL VARIATIONS OF ESTROUS CYCLE AND SEXUAL ACTIVITY OF OULED DJELLAL EWES IN ALGERIA

**Taherti Mourad*, Zidane Azdinia**

Laboratory of Natural Bio-Resources, Department of Biology, Faculty of Nature and Life Sciences, University Hassiba Benbouali of Chlef, PO Box 151, (02000) Chlef, Algeria

## ABSTRACT

This study aims to assess the reproductive potential and the particularities of sexual activity of the most important sheep breed in Algeria, called "Ouled Djellal". Data related to progesterone levels and estrous activity were monitored and followed up. The progesterone levels varied significantly ($P<0.05$) between ewes, and showed significant seasonal variations ($P<0.05$). The high levels of progesterone were observed from March to July (2.7±0.4 ng/ml), but low concentrations were recorded from October to February (1.4±0.4 ng/ml). Estrous activity followed similar variations to progesterone levels. Also, the highest total number of estrus was observed during the months of April to July. From September to February, the average duration of estrus recorded was 20±2.5 hours with more discreet heat signs, compared to 43±3 hours from March to August with more pronounced heat manifestations. The average length of estrous cycle in Ouled Djellal ewes was 18±0.6 days, with 70 % of the cycles between 16 and 21 days. From March to August the cycle length was 20±0.4 days. These results revealed that Ouled Djellal ewe shows different behavior than most breeds in Europe and North America: a sexual activity all year round with a decrease in autumn and an increase in spring/summer.

**KEYWORDS:**
Estrous cycle, ewes, Ouled Djellal breed, seasonal rhythm, pituitary gland, Environment

## INTRODUCTION

In Algeria, sheep farming in general, and potential of the breeds used in particular, are poorly known. Ouled Djellal breed alone represents 70% of the sheep flocks in Algeria. The remaining 30% are distributed over 9 other breeds, some of which are in clear regression (Hamra, Rembi and D'man) [1]. This meat breed known for its adaptation to the climatic conditions of the different breeding regions, is still not characterized and standardized. However, breeds evolving in harsh and highly changeable environments are likely to carry genes of interest controlling variations in behavioral and physiological traits to climatic hazards and farming conditions [2]. Therefore, the genotypes of these breeds can be very important for the development of sustainable livestock production systems, and the conservation of these local breeds has become a priority [3] Thus, this breed is attracting increasing interest in animal production development programs due to its perfect adaptation to different environments and its production qualities especially for meat [4,5]. However, the implementation of these programs is hampered by the lack of accurate data on the characteristics of reproduction. Most of the performance results for local breeds, although still limited, indicate factors of variation according to breed and environmental conditions [6,7,8]. The influence of environmental factors on reproduction were often reported in ewes [9], and the secretion of hormones is sensitive to variations in photoperiod [10] and temperature [11], playing a regulatory role in the endocrine mechanisms that control sexual activity in sheep [12].

Hence the interest of conducting this study, with the purpose to establish the characteristics of the estrous cycle and sexual activity of Ouled Djellal ewes, indicating the variations related to the breed and environmental conditions.

## MATERIAL AND METHODS

**Study area and environment.** The study was conducted at a sheep farm located 20 km from the city of Chlef (Algeria), between 36° 10′ North latitude and 1° 20′ East longitude and at 86 m altitude. The region is known for its Mediterranean climate of semi-arid type with mild, low-rainfall winters, and hot dry summers. Table 1 shows the average monthly variations in temperature, relative humidity and photoperiod of the region.

**Animal management.** The study involved 10 adult ewes; they were two to three years old and with an average weight of 52.5 ± 1.7 kg. The selected animals met the standard of the Ouled Djellal breed (physical characteristics and measurements). The

ewes were marked by tags and kept in loose housing, under seasonal variations of the environmental factors in the study area. They were fed with vetch-oat hay and 600 g of barley concentrate / animal / day, and watered *Ad libitum*. All animals were previously treated against classical sheep diseases and parasites.

**Data collection and studied parameters.** The blood samples were collected in the morning once a week and centrifuged within one hour. The serum obtained was stored at -20°C until hormonal analysis. Progesterone was analyzed according Elisa technique (Sheep progesterone Elisa kit RE 52231 96 - Ibl - International, Germany) [13]. According to Thimonier et al. (2000) [14] and Harouna Boureima et al. (2021) [15], we consider that ewe is cyclic from the threshold of 0.5 ng/ml.

On the other hand, during one year the animals were under twice-daily observation (morning and evening) of their sexual behavior in order to detect estrus, using rams with aprons that prevent intromission. Estrus is the duration between the first and last mating accepted by an ewe [16], the onset of estrus behavior is defined as the middle of the interval between the first control where heat was observed and the previous one [17], the end of estrus is the middle of the interval between the last observation of heat behavior and the next control and duration of estrus is defined as the time interval between two consecutive estrus starts [18].

**Data analysis.** The results obtained were expressed as means and standard deviations, graphs were realized by using Excel. The analysis of variance (ANOVA) and multivariate analysis (PCA) were achieved by using a trial version of Xlstat 2022.1.

## RESULTS

**Evolution of progesterone.** The progesterone levels varied significantly ($P<0.05$) between ewes, ranging from 0.1 ng/ml to 4 ng/ml. They showed significant seasonal variations: low from December to February (1.41 ± 0.42 ng/ml), then increased significantly ($P<0.05$) from April to August (2.68±0.25 ng/ml), with maximum values during June and July (2.42±0.16 ng/ml and 2.57±0.29 ng/ml), minimum values from September to February and a mean of 1.58±0.39 ng/ml (Figure 1).

**TABLE 1**
**Monthly means of temperature, humidity and photoperiod (length of day) during the study period**

| Months | Jan. | Feb. | Mar. | Apr. | May | Jun. | July | Aug. | Sep. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Temperature (°C)** | 11 | 11,4 | 14,7 | 14,5 | 23 | 27,9 | 30,3 | 30,5 | 25,9 | 21,2 | 13,4 | 10,6 |
| **Length of day (h)** | 9,75 | 10,83 | 11,93 | 12,5 | 13,93 | 14,5 | 14,33 | 13,6 | 12,5 | 11,7 | 10,2 | 9,83 |
| **Humidity (%)** | 79,3 | 67,1 | 66,7 | 71,5 | 51,5 | 49 | 40 | 41 | 52 | 62 | 72 | 78 |

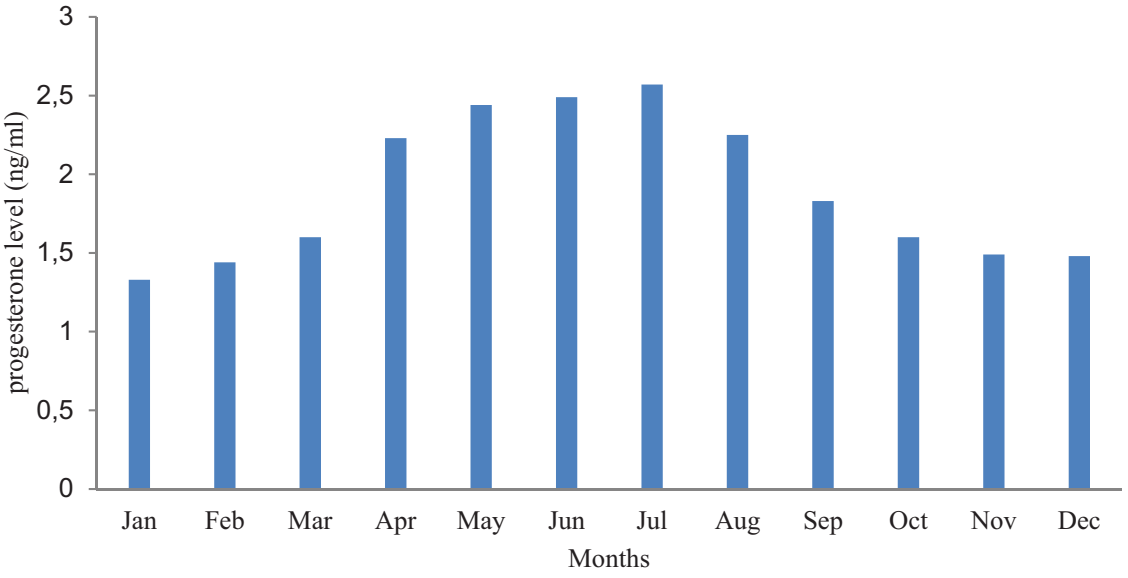



**FIGURE 1**
**Monthly variations of progesterone levels in Ouled Djellal ewes**

**Sexual characteristics of Ouled Djellal ewes. Estrus behavior.** Ouled Djellal ewes showed an estrus behavior in 90%. In the absence of rams in the flock, 2/10 ewes, showed frustrated signs of heat by riding their congeners. With the male, the most prominent signs observed in ewes in heat were energetic tail wagging and acceptance of mounting.

**Variation in number of ewes in estrus.** The proportion of ewes showing estrus behavior was 4/10 from December to February, reached 5/10 in March and 8/10 in July (Table 2). The highest total number of estrus was observed during the months of April to July. In this study, repetition of heat signs was recorded in some ewes, especially the youngest (2 years old) and with a satisfactory body condition (ewes 1 and 3).

**TABLE 2**
**Monthly variation of ewes showing estrus behavior and total number of estrus**

| Months | Jan. | Feb. | Mar. | Apr. | May | Jun. | July | Aug. | Sep. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of ewes in estrus | 4 | 4 | 5 | 6 | 7 | 7 | 8 | 6 | 6 | 6 | 5 | 4 |
| Total of estrus in ewes | 4 | 5 | 5 | 8 | 8 | 9 | 9 | 6 | 6 | 6 | 5 | 4 |

**TABLE 3**
**Length of estrous cycle and duration of estrus in Ouled Djellal ewes**

| Ewe | Age (year) | Weight (kg) | Duration of estrus (h) | Number of estrus | Length of estrous cycle (j) |
|---|---|---|---|---|---|
| **1** | 2 | 55.0±0.8 | 32.1±5.1 | 8 | 18.0±0.8 |
| **2** | 2 | 52.7±0.6 | 33.3±3.2 | 8 | 17.2±0.3 |
| **3** | 2 | 54.5±0.9 | 36.3±6,4 | 9 | 10.6±0.6 |
| **4** | 3 | 55.0±1.0 | 44,6±6.3 | 6 | 23.0±0.9 |
| **5** | 3 | 50.7±0.7 | 40.0±4.7 | 6 | 19.0±0.4 |
| **6** | 2 | 50.0±1.0 | 28.9±4.5 | 8 | 17.0±0.4 |
| **7** | 3 | 53.2±0.5 | 39.3±7.0 | 5 | 19.0±0.5 |
| **8** | 3 | 54.4±2.1 | 40.8±6.2 | 6 | 18.1±0.6 |
| **9** | 2 | 51.1±1,7 | 45.2±4.2 | 9 | 21.3±0.7 |
| **10** | 3 | 52.0±0.6 | 31.7±3.9 | 5 | 16.1±0.2 |
| **Mean** | 2.5±0.6 | 52.5±1.7 | 37.3±5.3 | 7.7 ±1.6 | 18.0±0.6 |

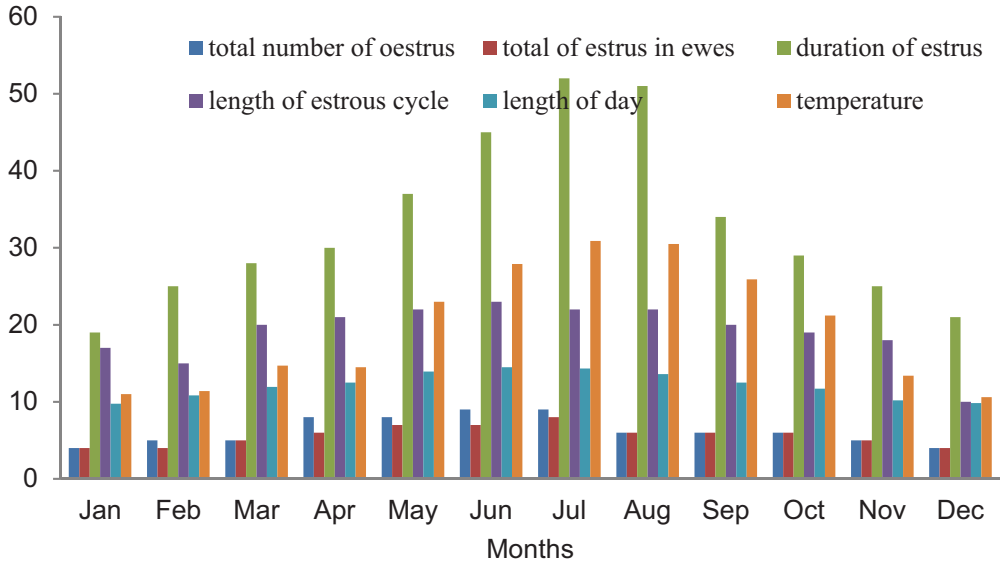



**FIGURE 2**
**Seasonal variations of the reproductive activity in Ouled Djellal ewes**

**Length of estrous cycle and duration of estrus.** The average length of estrous cycle in Ouled Djellal ewes from over 600 observations (60/ewe) was 18±0.6 days, with 70% of cycles between 16 and 21 days (Table 3). On the other hand, long and short cycle of 23±0.9 days (ewe 4) and 10.6±0.6 days (ewe 3) were respectively recorded in this work.

The average duration of estrus observed in this trial was 37.3±5.3 hours, with a minimum value of 27 hours and a maximum of 59 hours. 75% of heat events were observed in the morning (with acceptance of mounting). Duration of estrus was not significantly influenced ($P < 0.05$) by age and weight but rather by the month of the year. Indeed, from September to February, the average duration of estrus recorded was 20±2.5 hours with more discreet signs of heat, against duration of 43±3 hours from March to August with more marked manifestations of heat. Significant variations in the length of estrous cycle of Ouled Djellal ewes were recorded according to the seasons ($P < 0.05$), it was 15±0.7 days and 20±0.4 days respectively from September to February, and from March to August (Figure 2).

## DISCUSSION

Progesterone levels showed a clear increasing behavior during spring and summer season (March to July), during autumn and winter the levels were low (September to February). This pattern of progesterone levels variation corresponds to that of Peuth ewes in Niger, some sheep breeds in the Northern Hemisphere, Barbarine and Queue Fine de l'Ouest ewes in Tunisia and Ouled Djellal females in eastern Algeria, reported respectively by Wane (1989) [17], Thimonier et al. (2000) [14], Khaldi et al. (2011) [19] and Benyounes and Lamrani, (2013) [20].

The manifestations of estrus were discreet in Ouled Djellal ewes concerned by this study, in the absence of the male. The observed tail wagging was also mentioned describing the signs of estrus manifestation in the sheep species in general [21,22]. The scarcity of mounting between females, which is the typical sign of estrus in domestic cattle observed in the experimental flock (2% of ewes), was the same as that of Djallonké ewes in Benin and Congo [23,24] and Koundoum ewes and Oudah in Niger [25,26], which is partly responsible for the difficulties of heat detection in this species [27].

The average duration of estrus (37.3±5.3 hours) obtained in this study was in accordance to those obtained by several authors in other sheep breeds. For example, in Djallonke ewes in Burkina Faso, an average of 38.4±36.6 hours was reported by Boly et al. (2000) [28], and an average of 30.4±2.4 hours was recorded in desert ewes in Sudan and in ewes of tropical breeds [29,30]. The minimum duration of 27 hours obtained corroborates the variation in duration of estrus (27 to 59 hours) reported in tropical and inter-tropical breeds, cited by Charray and Havet (1983) [31], Yenikoye (1984) [18] and Álvarez and Zarco **(**2001) [32]. Similar lack of significant variability among ewes for duration of estrus due to age and weight was mentioned in the works of Boly et al. (2000) [28], Hanzen (2009) [33], Zongo and Meyer (2009) [34] in some sheep breeds, and Abdelsalam and Mohammed (2011) [35] in Awassi sheep from semi-arid regions of the Middle East.

The length of estrous cycle obtained in Ouled Djellal ewes in the present trial is included in the theoretical range of cycle lengths of 16 to 21 days reported in the literature for the sheep species [18,36,37]. This average duration in the sample studied is comparable to that of (18.3±0.7 days)obtained in local Oudah and Peul ewes from Niger by Yenikoye (2006) [38] and Gaillard (20017) [26], similar duration of (18.1±0.92 days) was reported in Djallonké ewes of Mossi variety in Benin by Hounzangbe-Adote (2014) [23] and in Burkina Fasso by Boly et al. (2000) [28]. Several authors like: Mbaye et al. (1990) [39]; Chikhi and Boudjenane (2003) [40]; Boudjenane (2006) [36]recorded equivalent durations of 18, 18.3 and 18.7 days respectively in Senegalese breeds and Beni Guil and Sardi ewes in Morocco.

Ouled Djellal ewes showed a reproductive activity over the year, with continuous estrus manifestations observed. The analysis of variance (ANOVA) revealed that the levels of progesterone, average number of estrus in ewes and average duration of estrus are highly significantly different ($p<0.0001$) between seasons (Table 4), they follow a parallel variation to the duration of day and the temperature of the study area, with maximum mean values in spring/summer (March to July) when the length of day is longest, and minimum mean values in shortest days of autumn/winter (September to February). Similarly, the longest average duration of estrus and estrous cycle of 43±3 hours and 20±0.45 days respectively, were recorded during the longest days (March to August). This implies that this mid-latitude breed does not seem to be influenced by photoperiod.

On the other hand, according to the principal components analysis (PCA), taking season into consideration (Figure 3), the first axis explaining 94.28% of the variance, and showing a clear separation between the seasons is positively correlated to summer and spring, but negatively correlated to autumn and winter. The analysis revealed that sexual activity in Ouled Djellal ewes is more pronounced during summer and spring seasons.

**TABLE 4**
**Analysis of variance of sexual activity parameters related to the season, showing the different groups and the p-value of each parameter**

| | Progesterone (ng/ml) | Number of ewes in estrus | Total of estrus in ewes | Duration of estrus (h) | Length of estrous cycle (j) |
|---|---|---|---|---|---|
| **Summer** | 2,4200 | 6,6667 | 8,6667 | 48,0000 | 22,3333 |
| **Spring** | 2,0667 | 6,0000 | 7,3333 | 30,4000 | 20,6667 |
| **Autumn** | 1,5567 | 5,3333 | 5,0000 | 28,8333 | 18,1667 |
| **Winter** | 1,3933 | 3,6667 | 3,6667 | 23,5333 | 15,3333 |
| **$R^2$** | 0,9909 | 0,8818 | 0,9580 | 0,9783 | 0,9015 |
| **Pr > F** | < 0.0001 | 0,0005 | < 0.0001 | < 0.0001 | 0,0002 |

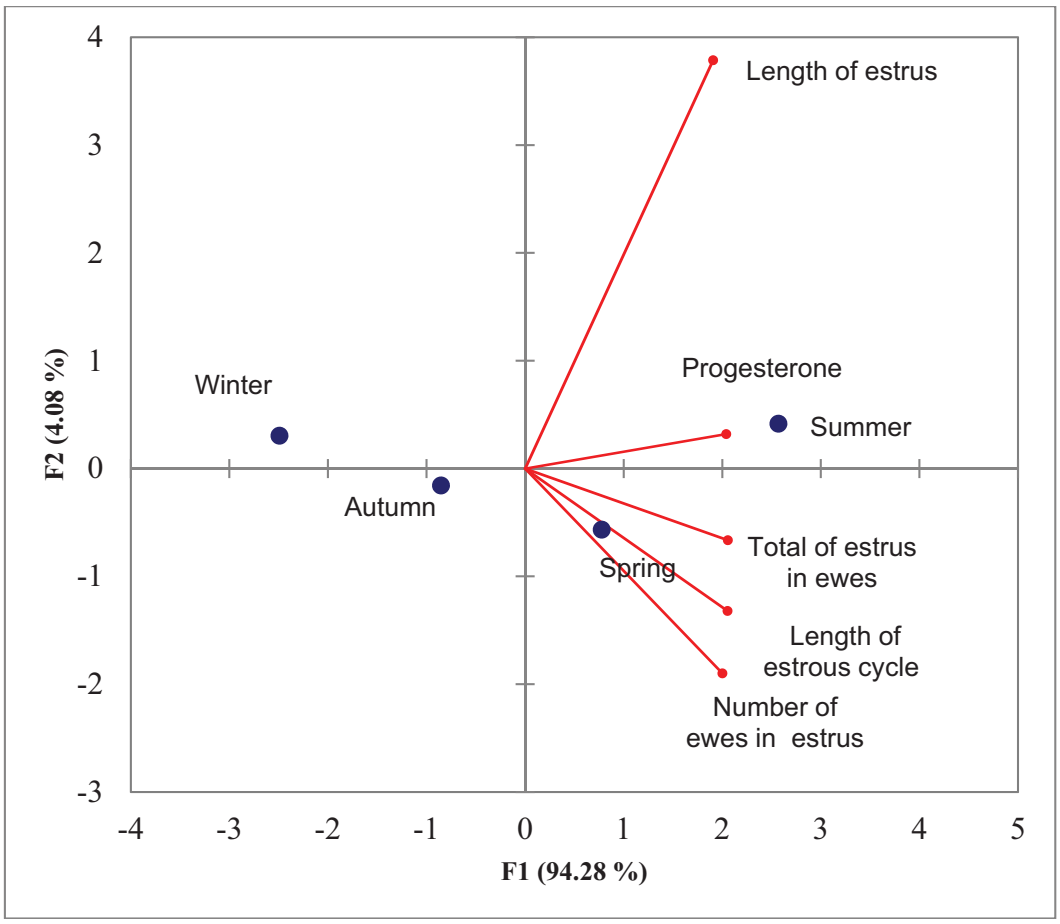



**FIGURE 3**
**Projection of variables on the first two axes of the PCA in Ouled Djellal ewes according to seasons**

Migaud et al. (2016) [12] reported that the majority of sheep breeds are polyestrous with a breeding season that extends from September to January for the northern hemisphere, this breeding seasonality exists in all sheep breeds in temperate or cold countries and the main intrinsic factor that modulates the sexual behavior of these breeds is the photoperiod, recognized as the necessary signal for the synchronization of sexual activity. Melatonin would be largely responsible for this characteristic of seasonality since its secretion, which is only nocturnal increases during the short days [41], acting as the primary transmitter of photoperiodic information [42]. According to Gaillard (2017) [26], this characteristic allows to these animals to give birth at a favorable time for the breeding of lambs, confirming that seasonality has completely disappeared or never existed in tropical and subtropical breeds. The signs of estrus observed in Ouled Djellal ewes throughout the year seem to confirm this thesis. Our observations are in line with those of Katherine (2008) [43] in Suffolk breed in Virginia at a latitude of 36°N (close to that of the region of Chlef), and Lassoued (1995) [44] in Barbarine and Thibar breeds in Tunisia, who showed that sexual activity is lower during autumn and winter months, and rises in spring and summer.

With the absence of a photoperiodic signal, sheep could use other environmental factors to synchronize their endogenous rhythm. Social contact between individuals (rearing in small groups) could

result in synchronization of reproductive status; the existence of ram effect in a group of females in anoestrus periods causes an increase in LH and induces sexual activity in females [45]. All these assertions can only reinforce the probable existence of an endogenous, light-independent rhythm in Ouled Djellal ewes, accentuated by the region's breeding systems, such as small-group breeding (family breeding) and the permanent presence of the ram with the ewes, a very common breeding practice in the study region.

In addition, temperature is one of the environmental factors that have a strong influence on the reproductive performance of sheep. For Ouled Djellal ewe, this study highlighted its adaptive capacity to local environmental conditions. It does not seem to be affected by high summer temperatures, on the contrary, the results showed a parallel evolution between its reproductive activity and the average monthly temperatures of its environment. Indeed, many authors have reported that in mid-latitudes (the case of the region of Chlef), the thermal environment is not one of the main drivers of sexual activity, and fluctuations in temperature do not alter the reproductive pattern of sheep [46].

## CONCLUSION

This research revealed that sheep reproduction in mid-latitude areas is different from that found in temperate high-latitude breeds. The results showed that sexual activity in Ouled Djellal ewes is continuous throughout the year, with varying proportions from one season to the next. From March onwards, the proportion of ewes with active ovaries rises to 50%, reaching 80% in July, compared with 40% in winter. This seasonal distribution confirmed that ovarian activity in Ouled Djellal ewes is higher when the photoperiod is increasing (spring and summer) than when it is decreasing (autumn and winter). Ouled Djellal breed is also little affected by high summer temperatures, that is not in line with the conclusions generally put forward for sheep, which are considered to be sexually short-day species where photoperiod plays an essential role in regulating seasonal reproductive activity.

The importance of estrus control remains in the fact that it is an operation conditioning the profitability of the farms, indeed, the acquisition of observations and the knowledge of ewe's behavior during estrus should make it possible to obtain references likely to help in the determination of the optimal period of insemination and consequently improve fertility. The lack of marked seasonality in Ouled Djellal breed can be an asset to optimize the productivity and the reconstitution of the herds, as part of the implementation of programs of conservation and improvement of this breed.

## ACKNOWLEDGEMENTS

The authors warmly thank the technical staff of the sheep farm for their invaluable assistance during the experiment.

## REFERENCES

[1] Laoun, A., Harkat, S., Benadji, R., Yabrir, B., Hakem, A., Ranebi, D., Maftah, A., Madani, T., Dasilva, A., Lafri, M. (2015) Phenotypic characterization of the Rembi sheep breed from Algeria. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux.(in French). 68(1), 19-26.

[2] Barker, J.S.E. (1999) Conservation of livestock breed diversity. (in French). Agriculture. 25, 33-43.

[3] Boisson de Chazournes, L. (2009) Convention on biological diversity and its protocole on biosafety. In United Nations Audiovisual Library of International Law, May 22, 2009, Geneva. 1-9.

[4] Sadoud, M. (2011) Place of the butcher's activity in the Algerian red meat sector. Archives of Zootecnia. 60(230), 309-312.

[5] Benyounes, A., Lamrani, F., Benyounes, A. (2015) Carcass quality of Ouled Djellal lambs fattened under different medico-food diets. Revue Agriculture.(in French). 9, 4-9.

[6] Taherti, M. and Kaidi, R. (2018) Reproductive performance and numerical productivity of Ouled Djellal ewes. Lebanese Science Journal.(in French). 19(1), 47-58.

[7] Belhadia, M., Fantazi, K., Benaouina, H., Kada, M., Belhandouze, T., Housseini, N. (2020) Reproductive performance of Taadmit ewes with male effect and heat synchronization. Livestock Research for Rural Development.(in French). 32(8).

[8] Zidane, A., Taherti, M., Gadouche, L., Metlef, S., Ababou, A. (2021) Seasonal variations in the reproductive performance of Ouled Djellal ewes in the region of Chlef, Algeria. Revue d'Elevage et de MédecineVétérinaire des Pays Tropicaux. (in French).74(4), 193-198.

[9] Chemineau, P., Malpaux, B.,Brillard, J.,Fostier, A. (2010)Photoperiod treatments and reproduction in farm animals. Bulletin de l'Académie Vétérinaire de France. (in French).163(1), 19-26.

[10] Meyer, C. (2009). Photoperiod and melatonin in domestic herbivores. Bibliographic Note, April 2009, CIRAD, Montpellier, France. (in French).1-22.

[11] Phogat, J.B., Pandey, A.K., Singh,I. (2016) Seasonality in buffaloes reproduction. International Journal of Plant Animal and Environmental Sciences. 6(2).

[12] Migaud, M., Dardente, H., Keller, M., Batailler, M., Meurisse, M., Pillon-Ricaud, D. (2016) Neuroendocrine control of reproduction in mammals. INRAE Productions Animales. (in French).29(4), 255-266.
[13] Saidani, F., Slimane, N., Khaldi, S., Chettoui, C. (2012) Assays of progesterone and PSPB for the monitoring and analysis of the results of the insemination of dairy cows in the mountainous and forest areas of northwestern Tunisia. Revista Electronica de Veterinaria. (in French).13(10), 1-18.
[14] Thimonier, J., Cognie, Y., Lassoued, N., Khaldi, G. (2000) The male effect in sheep: a current technique for controlling reproduction. INRAE Productions Animales.(in French). 13, 223-231.
[15] Harouna Boureima, K., Issa Ibrahim, A., Mani, M., Arichatou, H. (2021) Maradi red goat sexual cycle: descriptive and progesteronomic study. International Journal of Biology and Chemical Science. 15(1), 26-33.
[16] Castonguay, F. (2018) Reproduction in sheep. Edition March 2018, Department of Animal Sciences Laval University, Quebec, Canada.(in French) 26-33.
[17] Wané, A. (1989) Study of the characteristics of the sexual cycle in Senegalese ewes of the Djallonke, Touabire and Peulh breeds by radiolmunodosage of progesterone, University of Dakar, Senegal.(in French).80.
[18] Yenikoye, A. (1984)Annual variations in estrus behavior, rate and possibility of ovulation in Peulh ewes from Niger. Reproduction Nutrition Development. (in French).24, 11-19.
[19] Khaldi, Z., Haddad, B., Souid, S., Rouissi, H., Ben Gara, A.,Rekik, B. (2011) Phenotypic characterization of the sheep population of southwestern Tunisia. Journal of Animal and Genetic Research.(in French).49, 1-8.
[20] Benyounes, A. and Lamrani, F. (2013) Seasonal anestrus and sexual activity in Ouled Djellal ewes. Livestock Research for Rural Development.(in French). 25(8).
[21] Malpaux, B., Wayne, N., Karsch, F.J. (1988) Termination of the breeding season in the Suffolk ewe: involvement of an endogenous rhythm of reproduction. Biology of Reproduction. 39, 254-263.
[22] Bocquier, F., Gaubert, J., Blanc, F., Viudes, G., Maton, C., Debus, N., Teyssier, J. (2006) Use of electronic identification for automated detection of sexual behavior in sheep: prospects for heat detection in sheep. Rencontres RecherchesRuminants.(in French). 155-158.
[23] Hounzangbe-Adote, M.S. (2014) Study of the estrous cycle in the Djallonké ewe. FAO, Document.(in French). 1-10.
[24] Missoko, R., Akouango, P., Ognika, A., Mbabala, G.R.J. (2020) Reproduction parameters of Djallonké sheep in a village environment in the pool department (Congo Brazzaville). Journal of Animal and Plant Science. (in French).46(1), 8141-8148.
[25] Hamadou, I., Moula, N., Mani, M., Issa, M., Antoine-Moussiaux, N., Farnir, F., Leroy, P., Marichatou, H. (2015) Contribution to the study of the characteristics of the estrous cycle in the ewe and the sperm characteristics in the ram of the Koundoum breed, in Niger. Revue de MédecineVétérinaire.(in French). 166(3-4), 113-120.
[26] Gaillard, Y. (2017) Characteristics of the reproduction of the Oudah sheep. Revue d'Elevage et de MédecineVétérinaire des Pays Tropicaux.(in French). 32(3), 285-290.
[27] Yahimi, A., Djellata, N., Hanzen, C., Kaidi, R. (2013) Analysis of heat detection practices in Algerian dairy cattle farms. Revue d'Elevage et de MédecineVétérinaire des Pays Tropicaux. (in French).66, 31-35.
[28] Boly, H., Peneme, M., Swadogo, L., Sulon, J., Beckers, J., Leroy, P.L. (2000) Effect-dose-response of gonadotropin (PMSG) on the reproduction of the Djallonké ewe 'Mossi' variety. Tropiciculture.(in French). 18(3), 126-129.
[29] Godfrey, W.R. and Hensley, E.L. (1999) Estrus synchronization and artificial insemination of hair sheep ewes in the tropics. Theriogenology. 51(5), 985-997.
[30] Makawi, S.A. and Manahil, Z.A. (2007) Fertility response of deserts ewes to hormonal estrous synchronization and artificial insemination using Fresh diluted semen. Journal of Animal and Veterinary Advances. 6(3), 385-391.
[31] Charray, J. and Havet, A. (1983) Reproductive performance of West African dwarf ewes maintained in the central region of Ivory Coast. International meeting on ruminant reproduction in the tropics, Guadeloupe, June 8-10, 1983,(in French).1-10.
[32] Álvarez, R.L. and Zarco, Q.L.A. (2001) Male and female stimulation of estrous activity in sheep and goats.Veterinary México. 32(2), 117-129.
[33] Hanzen, C. (2016) Detection of estrus in ruminants. Bibliographic note. Faculty of veterinary medicine, University of liège, Belgium.(in French). 1-22.
[34] Zongo, M. and Meyer, C. (2009) Seasonal variations in sheep reproduction. Annals of the University of Ouagadougou. (in French). 7, 1-25.
[35] Abdelsalam, Q.T. and Mohammed, M.A. (2011) Awassi sheep reproduction and milk production. Tropical Animal and Health Production. 43, 1319-326.

[36] Boudjenane, L. (2006) Reproduction and production performance of Moroccan sheep breeds. Perspectives in Agriculture, Veterinary Science, Nutritional and Natural Research. 1(014), 1-18.
[37] Derouin-Tochon, F., Beltramo, M., Decourtc,M.,Fleurot, R., Gérard, N.,Pinet-Charvet, C., Martinet, S., Robert, V., Taragnat, C., Tillet, Y., Duittoz, A. (2019) Ovulation in mammals. INRAE Productions Animales. (in French).32(3), 445-460.
[38] Yenikoye, A. (2006) Studies of sexual endocrinology and follicular growth in Nigerien sheep of the Peul breed: influence of the breeding season, François Rabelais University, Tours, France. (in French).189.
[39] Mbaye, M., Diop, P.E.H, Wone, A. (1999) Characteristics of the sexual cycle in Senegalese sheep of the Touabire, Peul and Djallonke breeds, Senegalese Agricultural Institute. (in French). 90.
[40] Chikhi, A. and Boujenane, L. (2003) Zootechnical characterization of Sardi breed sheep in Morocco. Revue d'Elevage et de MédecineVétérinaire des Pays tropicaux.(in French). 56 (3-4), 187-192.
[41] Chalivoix, S. (2010) Photoperiod transition and neuronal plasticity in the ovine hypothalamus: neuralatomic and functional aspects, François Rabelais University, Tours, France. (in French).155.
[42] Brunet, A.G., Santiago, J., Moreno, J., Del Campo, A., Malpaux, B., Chemineau, P., Bulnes, D.J, Sebastia, A.L. (2008) Endogenous circannual cycles of ovarian activity and changes in prolactin and melatonin secretion in wild and domestic female sheep maintained under a long-day photoperiod. Biology and Reproduction. 78, 552-562.
[43] Katherine, M.J. (2008) Characterization of seasonal reproduction in Virginia Tech selection Line, St. Croix, and Suffolk ewes, University of Virginia, USA. 140.
[44] Lassoued, N. and Khaldi, G. (1995) Seasonal variations in sexual activity in Western thintail and black Thibar ewes. In: sheep farming in arid and semi-arid zones. In: CIHEAM, Cahiers Options Méditerranéennes, 1995, Zaragoza, Spain, 27-34. (in French).
[45] Wayne, N.L., Malpaux, B., Karsch, F.J. (1989) Social cues can play a role in timing onset of the breeding season of the ewe. Society for Reproduction and Fertility. 87, 707-713.
[46] Sawyer, G. and JitikNaravan, E. (2019) Chapt 2.A review of the influence of climate chang on sheep reproduction. In: Comparative Endocrinology of Animal, editions Narayan Jitik E., University of Queensland, Australia. 80-125.

**Received:** **29.07.2023**
**Accepted:** **12.08.2023**

## CORRESPONDING AUTHOR

**Taherti Mourad**
Laboratory of Natural Bio-Resources
University Hassiba Benbouali of Chlef
Chlef 02000 – Algeria

e-mail: tahmourad.2023@gmail.com

-© by PSP Volume 32– No. 09/2023 pages 2981-2987 Fresenius Environmental Bulletin

# PHYSICO-CHEMICAL QUALITY OF WATERS OF TWO DAMS IN EASTERN ALGERIA KOUDIAT MEDOUAR (BATNA) AND BABAR (KHENCHLA)

**Menasria Amel*, Allalgua Amel, Kaouachi Nouha**

Laboratory of Terrestrial and Aquatic Ecosystems, Faculty of Natural and Life Sciences, University of Mohamed Cherif Messaadia, BP 1553, Annaba Road, 41000 Souk Ahras, Algeria

## ABSTRACT

Water quality is an important parameter that affects all aspects of ecosystem and human well-being such as community health, food production, economic activities, ecosystem health and biodiversity.The objective of our work was to study the evolution of physico-chemical parameters of surface waters of the two dams of eastern Algeria (Koudiat Medouar and Babar), based on analytical results of the ANRH during a period from january to december 2020.

Analyses were carried out on 10 physico-chemical parameters; descriptive analysis of the results showed that the physico-chemical qualities of water from the Koudiat Medouar and Babar dams comply with Algerian standards for raw water.

The results have been treated statistically by using the major issue analysis (PCA). The aim of this multivariate modelling is to determine the main factors controlling the chemistry of these waters and possible sources of contamination and pollution.

Our results reveal that the dam waters Koudiat Medouar show a strong negative correlation with OM, $DBO_5$, $NO_2$-, DR, COD, $PO_4^{-3}$. This is linked to industrial and agricultural effluents and the discharge of domestic wastewater.

**KEYWORDS:**
Koudiat Medouar dam, Babar dam, physico-chemical parameters, PCA

## INTRODUCTION

Water is of vital biological and economic importance. The hydrosphere is the foundation of life and ecological balance. Water is a foodstuff, possibly a medicine, a raw material for industry, energy and agriculture, and a means of transport. Its uses are therefore manifold, but when it comes to human health, they are dominated by agriculture and aquaculture, industry and crafts, aquatic leisure activities including swimming, and above all the collective or individual supply of drinking water, used for food purposes (drinking water, cooking) but also for domestic and hygiene purposes [1].

Surface water resources are of vital importance to Algeria's socio-economic development. Since the 1980s, surface water has been increasingly mobilized by means of hydraulic dams. In total, out of a total of 74 dams in operation (situation year 2015), 25 dams are located in the Northeast and control an overall drained area of 25,224 km² [2]. However, the availability of water per capita is likely to be increasingly reduced if some of these resources are rendered unusable by pollution. For this reason, it is essential to monitor water quality, which can be assessed by physical, chemical and biological analyses [3].

In Algeria, dams are used for both drinking water supply and irrigation. In fact, these freshwater resources are exposed to various types of pollution of multiple origins: industrial, urban and agricultural, generating damage for man and his environment (fauna and flora).

The aim of this study is to assess the physico-chemical quality of the water in the Babar dam (Khenchela wilaya). The latter was built to meet the drinking water needs of the towns of Babar, Chechar, Ain Djerbaou, Bouhmama and Tamza, and to irrigate farmland downstream of the dam, as well as the Koudiat Medouar dam in Timgad (Batna wilaya), which was built to boost agriculture in the highlands of eastern Algeria, and to produce drinking water for the city of Batna and its neighbors (Khenchela, Oum el Bouaghi and Biskra).

## MATERIALS AND METHODS

**Study Area.** The field of this study concerns two freshwater dams (Babar and Koudiat Medouar), distributed over a vast region of eastern Algeria.The Babar dam (35°10′32″ N, 7°2′14″ E) is located in the wilaya of Khenchla 30 km from the said city and 9 km south-west of the city of Babar. With a capacity of 41 million $m^3$, the Babar dam plays an essential role in supplying drinking water to the populations of neighboring areas (Ouldja, Khirane, Djellal, Chechar) up to 60% of needs, as well as for the irrigation of agricultural land [4]. The Koudiet Medouar dam (35°30′57″ N, 6°30′48″ E) is situated on the

Oued Reboua at 35 kilometers from the chief town of Batna. It is part of the Beni-Haroun complex system [5]. The reservoir has the capacity of 74 million $m^3$, intented for the supply of drinking and industrial water to the willaya of Batna, Khenchela and Ain Touta, as well as the irrigation of more than 15700 ha of agricultural land in the plains of Batna, Chemora, Ain Touta and El Kantara [5].

**Sampling and physicochemical analyzes.** In order to have a good knowledge of the water quality of Koudiat Medouar and Babar dams, it is important to study the variation of several chemical elements that are at the origin of a contamination or a pollution industrial, urban or agricultural.For this, we used the agency's database of chemical analyses of the National Agency of Hydraulic Resources (ANRH) of Constantine during the period from january to december 2020.These are hydrogen potential (pH), dry residue (DR), dissolved oxygen (DO), nitrates ($NO_3^-$), nitrites ($NO_2^-$), ammonium ($NH_4^+$), phosphate ($PO_4^{-3}$), organic matter (OM), chemical oxygen demand (COD) and biological oxygen demand ($BOD_5$).

Physico-chemical characterization (m±ESM), principal component analysis (PCA), as well as analysis of the correlation were obtained with the help of the STATISTICA software (StatSoft, version 10) for Windows.

## RESULTS AND DISCUSSION

**Physicochemical characteristics of water.** The values of the global parameters of the water quality of the two dams (Babar and Koudiat Medouar) shown in Table 1.

**Hydrogen potential (pH).** Corresponding to the concentration of hydrogen ions, pH is used to measure the acidity or basicity of water [6]. The analysis of the pH results does not present any notable difference between dams during the four seasons, and tend to be basic with a minimum of 7,43 at Babar dam and a maximum of 7,81 at Koudiat Medouar dam. The physical environment characterized in part by limestone would probably be at the origin of this alkalinity [6,7].This low alkalinity has been recorded also by [8] in the Koudiat Medouar dam and by [9] in the Babar dam.

**Dry residue (DR).** According to [10], dry residue is the amount of solid matter in water, i.e. the sum of matter in solution and in suspension. The latter give the water its brownish color and sometimes dark, which conditions the penetration of light in the environment and thus influences the aquatic fauna and flora. The inter-dam variation in dry residue content shows that the dams Koudiat Medouar and Babar are characterized by average values of 791,33±55,42 ; 699,33±46,08 ; respectively. Thus, the waters of these two dams are characterized by a water "rich in minerals" (calcium, magnesium and/or sodium). These results are consistent with those obtained by [8] in the dam of Koudiat Medouar and by [11] in Beni Haroun dam.

**TABLE 1**
**Seasonal variation of physico-chemical parameters in water at Babar and Koudiat Medouar dams during the period january to december 2020 (m±SEM, n= 1)**

| Dams | Babar | | | | K.Medouar | | | |
|---|---|---|---|---|---|---|---|---|
| Season | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn |
| pH | 7,72±0,52 | 7,43±0,34 | 7,75±0,18 | 7,6±0,17 | 7,81±0,10 | 7,54±0,27 | 7,71±0,13 | 7,69±0,20 |
| DR (mg/l) | 670±85,51 | 695,33±43,09 | 716±17,32 | 716±8 | 794±43,86 | 760,66±30,02 | 760,66±30,02 | 868±4,16 |
| DO (%) | 93,27±2,76 | 95,33±11,14 | 98,56±2,07 | 94,71±1,59 | 90,76±3,20 | 96,48±7,52 | 101,69±5,51 | 97,06±0,90 |
| $NO_3^-$ (mg/l) | 0,33±0,57 | 1,66±2,08 | 0,33±0,57 | 2,66±2,51 | 0,66±0, 57 | 3,66±1,52 | 2,66±2,30 | 3±3,60 |
| $NO_2^-$ (mg/l) | 0,02±0 | 0,02±0 | 0,01±0,01 | 0,01±0,01 | 0,09±0,04 | 0,10±0,01 | 0,12±0,01 | 0,08±0,03 |
| $NH_4^+$ (mg/l) | 0,04±0,04 | 0,02±0,01 | 0,03±0 | 0,01±0,01 | 0,02±0,01 | 0,033±0,01 | 0,04±0,01 | 0,02±0,02 |
| $PO_4^{-3}$(mg/l) | 0,03±0,02 | 0,03±0,01 | 0,01±0 | 0,01±0,01 | 0,05±0,02 | 0,02±0,01 | 0,01±0 | 0,08±0,03 |
| OM (mg/l) | 5,26±0,65 | 5±0,52 | 4,56±0,11 | 4,4±0,86 | 7,53±0,11 | 7,2±0,2 | 6,76±0,75 | 7,5±0,86 |
| COD (mg/l) | 24,66±0,57 | 22,66±4,04 | 22,66±2,88 | 18,66±0,57 | 26±1 | 25,33±0,57 | 28±1,73 | 23,66±1,15 |
| $BOD_5$ (mg/l) | 1±0 | 1,66±0,57 | 1,66±0,57 | 3,33±0,57 | 1,33±0,57 | 3±1 | 3±0 | 5,33±0,57 |

**Dissolved oxygen (DO).** Dissolved oxygen is one of the main factors in the nitrogen cycle. Its presence or absence is decisive for the transformation processes of mineral forms of nitrogen [12]. The concentrations of dissolved oxygen, constitute with the pH values, one of the most important water quality parameters for aquatic life [13]. The levels of dissolved oxygen levels during our study show a similarity between the two dams. The highest values are recorded during the warm season (summer), which would be related to the chlorophyll function of aquatic plants, which increases the dissolved oxygen content of the water and can cause oxygen saturation phenomena ([14, 15]. Oxygen saturation levels in the four dams are mostly average to good. This is related to the large surface area of the dams lakes which allows a better oxygenation of the environment, and the high precipitation of these regions.Compared to other environments, our dissolved oxygen levels are higher than those recorded by [16] at the Foum El Khanga dam,and are consistent with those found by [11] at the level of Beni Haroun dam waters.

**Nitrates ($NO_3^-$).** They represent the most oxygenated form of nitrogen. It's a highly soluble form. Its presence is linked to the intensive use of chemical fertilizers **[**17]. Seasonal variations in $NO_3^-$ levels show a maximum value at Koudiat Medouar dam during spring, with an average of 3,66±1,52 mg/l, followed by Babar dam during autumn, with an average of 2,66±2,51 mg/l. The origin of the important quantities of nitrates comes mainly from the application of chemical fertilizers during fertilization [18]. In case of rain, the nitrate ions that are not assimilated by the plants are washed into the dams [10]. Nevertheless, its values remain lower than the limit value of 50 mg/L set by the Algerian standards [19]. Compared to other environments, our nitrate contents are largely inferior to those recorded by [20] at the level of the Cheffia and Mexa dam.Some authors who have worked on rivers have shown that the $NO_3^-$ content is higher during the wet season than during the dry season, as shown by our results [21].

**Nitrite ($NO_2^-$).** Nitrite ions ($NO_2^-$) are present in the soil, in natural waters and in plants, but in relatively small quantities [6].The determination of nitrites in the waters of the two dams allows to record low values not exceeding the standard of the WHO (2002) (0,1 mg/L). These low levels of nitrite show that these particles come mainly from the metabolism of nitrogen compounds; they are part of the nitrogen cycle between ammonia and nitrates. Our nitrite contents are close to those obtained by [22] at the level of Wadi Fez and by [4] at the level of Babar dam.

**Ammonium ($NH_4$+).** In the case of ammonium ion, whose origin is essentially urban [7]. The concentrations are all below 0.5 mg·L-1 (Table 1), the limit value recommended by the European Union [6].The low levels of ammonia ion show that these particles come mainly from the metabolism of nitrogen compounds where ammonium is transformed rather quickly into nitrites and nitrates, by bacterial oxidation and do not indicate any pollution of anthropic origin . Our results remain comparable with those found by [23] at the Sidi Chahed dam.

**Phosphate ($PO_4^{-3}$).** The dosage of orthophosphates in the two dams gives very low values. The concentration fluctuated between 0,01 mg/l and 0.03 mg/l for the waters of the Babar dam, and between 0,01 mg/l and 0,08 mg/l for the waters of the Koudiat Medouar dam. This level of orthophosphates is linked to the absence or very low use of phosphate fertilizers, as well as inputs of phosphate detergents or lyes and detergents or phosphate lyes and human dejecta. Our results remain comparable to [24].

**Organic matter (OM).** Surface waters naturally contain organic matter made up of decomposition products organic matter consisting of decomposition products of animal or plant origin, elaborated under the influence of micro-organisms [25]. The contents of the organic matter estimated by the KMnO4 are quite low at the level of the Babar dam during the whole study period. Such a result could be due to phenomena of biodegradation of organic substances following the conditions of oxygenation which allow a perfect decomposition of the organic load. Our results are consistent with those of [25] on the waters Ain Dalia and Zit El-Anba dams.At the level of Koudiat Medouar dam, we recorded important values during autumn (7,5 mg/l) and winter (7,53 mg/l), translating a strong organic load which can be due to an enrichment water in organic matter following heavy rains or floods. The lowest content was reported during the summer season (6,76 mg/l), reflecting a return to an initial situation, following a good biodegradation of the organic substances discharged at the Koudiat Medouar dam.

**The biological oxygen demand ($BOD_5$).** $BOD_5$ values for the observation period, range from a maximum of 5,33mg/l (at the Koudiat Medouar dam) and a minimum of 1 mg/l (at the Babar dam). During this period, the increase in $BOD_5$ levels in the waters of the Koudiat Medouar dams, reflecting the increase in the pollutant load due to anthropogenic action. According to **[**15], the $BOD_5$ is a measure of biodegradable organic carbon, and under certain conditions of the reduced nitrogenous forms in wastewater. Our results support those of [9] on the same study sites

**The chemical oxygen demand (COD).** At the water level of the two dams, the average values of COD far exceed those of $BOD_5$. They varied between 18,66 mg/l (during autumn) and 24,66 mg/l

(during winter) for the Babar dam, and between 23,66mg/l (during autumn) and 28mg/l (during summer) in the waters of the Koudiat Medouar dam. This state of affairs testifies to the predominance of pollution of various origins (industrial, agricultural, urban).In fact, these rather high COD contents compared to those of the $BOD_5$, could certainly reflect the presence of a low biodegradable load dominated by micropollution (heavy metals), possibly masked by the organic load [26]. These results are comparable to those of [8].

**Principal component analysis of measured parameters.** The PCA was applied to 10 physico-chemical parameters measured in the surface waters of 2 dams (pH, DR, DO, $NO_3^-$, $NO_2^-$, $NH_4^+$, $PO_4^{-3}$, OM, $BOD_5$, COD). The purpose of this multivariate modeling is to determine the main factors that control the chemism of these waters and the possible sources of contamination and pollution

Regarding the PCA results, the first two axes of the analysis express 54,31% of the total inertia included in our hydrochemical data matrix, this means that the maximum of the total variability is represented by the F1vs F2 factorial (Figure 1).

The 1st axis alone explains 37,31% of the inertia and is strongly correlated (negatively) with the following variables : OM (r=-0,95), $BOD_5$ (r=-0,85), $NO_2^-$ (r= - 0,78), DR (r= -0,76), COD (r=-0,59), $PO_4^{-3}$ (r= - 0,58) (Table 2).

This cluster therefore includes most of the parameters that determine the organic and domestic pollution indicators. This axis defines an increasing gradient of organic pollution, going from its positive pole to the other negative pole.However, DO (r= +0,09), $NH_4^+$ (r= +0,11) are positively correlated to this principal component. This axis then defines a gradient of pollution mainly due to the ammonification of nitrogenous substances.

The in-depth analysis of the structuring of the PCA factorial plan for the "dam" facteur, clearly shows that the 1st axis stipulates the presence of two clusters, the first includes the Koudiat Medouar dam and the second includes the Babar dam.

In general, the 1st axis shows that the surface waters of the Koudiat Medouar dam are characterized mainly by high values of the parameters : Hydrogen potential, nitrates, nitrites,phosphate ,chemical oxygen demand, dry residue and lower values for organic matter and biological oxygen demand. This is linked to industrial and agricultural effluents and the discharge of domestic wastewater.

While the Babar dam is characterized by higher values of dissolved oxygen and ammonium ion. This means that the waters of Babar dam present a gradient of nitrogenous pollution could derive from industrial and urban wastewater.

## CONCLUSION

The present work is part of the evaluation of the physico-chemical quality of the waters of two dams of Eastern Algeria (Babar and Koudiat Medouar), are used both for drinking water supply and for irrigation.

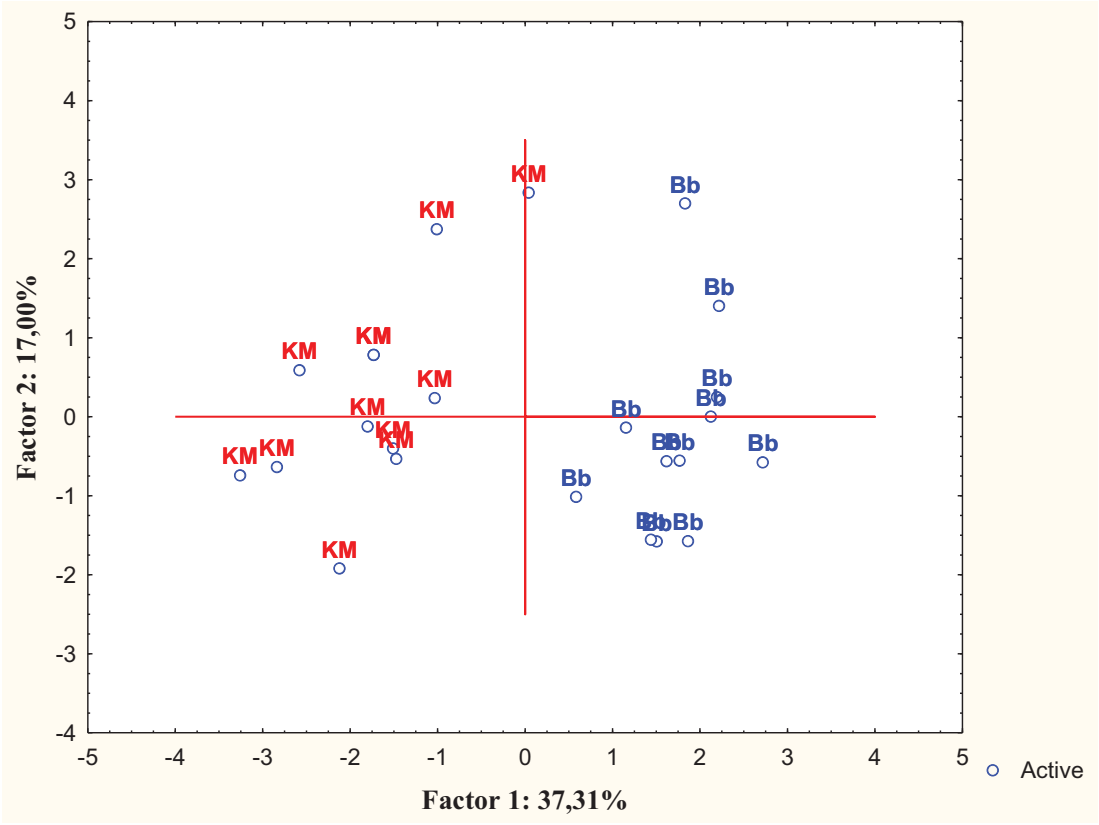
In the light of the results obtained, we note that almost all the parameters analyzed of the waters of the two dams Koudiat Medouar and Babar are in conformity with both the national and international regulations in terms of water potability, with the exception of one of the parameters of pollution reflecting an important degree marked by an increase in COD contents, which means a water of fair to poor quality.

These results were subjected to a Principal Component Analysis (PCA).The ultimate aim of which is to interpret the results obtained, to highlight the correlation between the various parameters and the differentiation of the two dams according to their physico-chemical characteristics.

**TABLE 2**
**Principal component analysis: relationships between physico-chemical parameters and PCA axes**

| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | -0,186 | **-0,461** | 0,357 | -0,688 | -0,246 | 0,264 | -0,087 | 0,072 | 0,042 | -0,075 |
| DR | **-0,766** | -0,256 | 0,426 | 0,144 | -0,297 | 0,135 | 0,024 | -0,072 | -0,109 | 0,139 |
| DO | 0,098 | **0,749** | 0,478 | 0,057 | -0,353 | -0,140 | 0,027 | 0,222 | 0,035 | 0,014 |
| $NO_3^-$ | 0,374 | -0,021 | **-0,637** | 0,440 | -0,347 | 0,328 | -0,100 | 0,137 | -0,010 | -0,030 |
| $NO_2^-$ | **0,782** | 0,257 | -0,177 | -0,238 | -0,109 | -0,369 | -0,265 | -0,055 | -0,095 | -0,042 |
| $NH_4^+$ | 0,116 | **0,822** | 0,086 | -0,170 | 0,218 | 0,443 | -0,114 | -0,121 | -0,030 | 0,011 |
| $PO_4^{-3}$ | **0,581** | 0,177 | 0,348 | 0,253 | 0,626 | 0,054 | -0,100 | 0,194 | -0,042 | -0,017 |
| OM | **0,950** | 0,090 | 0,123 | -0,053 | 0,093 | -0,027 | -0,047 | -0,037 | 0,225 | 0,074 |
| COD | **0,598** | 0,212 | -0,449 | -0,531 | 0,143 | 0,023 | 0,265 | 0,116 | -0,074 | 0,031 |
| $BOD_5$ | **0,855** | 0,174 | 0,263 | 0,288 | -0,067 | 0,036 | 0,208 | -0,123 | 0,009 | -0,145 |

**Values in bold are different from 0 at significance level alpha=0.05**



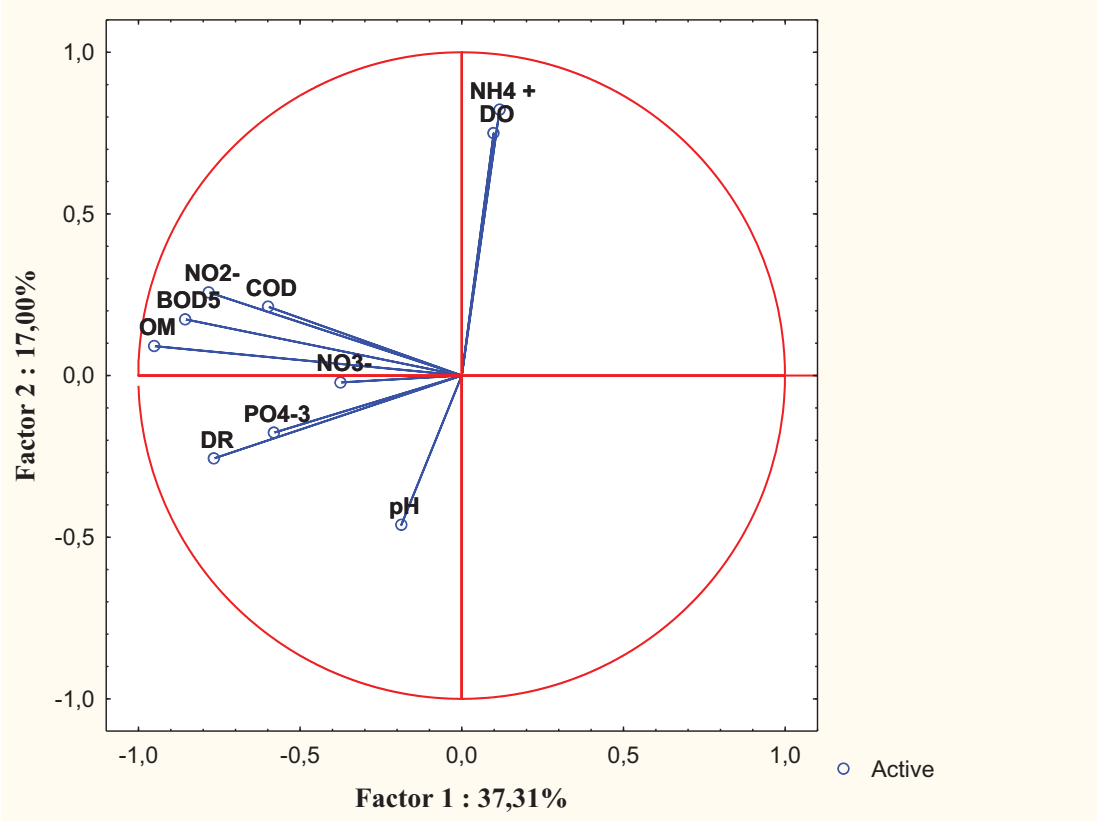




**FIGURE 1**
**Distribution of physico-chemical parameters and dams on plane *F1 x F2*.**

The standardized principal component analysis allowed us to differentiate between a zonality of water quality in the dams studied. In fact, the PCA enabled us to clearly distinguish between the waters of the two dams, and also to describe the structure by two main characteristics:

The waters of the Koudiat Medouar dam ares characterized by organic pollution which results in values of hydrogen potential, nitrates, nitrites,phosphate ,chemical oxygen demand and dry residue.

The waters of the Babar dam ares characterized by nitrogenous pollution, given the values of dissolved oxygen and ammonium ion.

## REFERENCES


[1] Festy, B., Hartemann, P., Ledrans, M., Levallois, P., Payment, P., Tricard, D. (2003) Water quality. In Environnement and Public Health-Fondations and Practices. 333-368. (In French).

[2] Boutouatou, F., Mebarki, A., Nouaceur, Z., Laignel, B. (2019) Eastern Algerian dams: Characterization, water balances and hydrological Variability. Sciences & Technologie. 50, 9-23. (In French).

[3] Afri-Mehennaoui, F.Z., Mehennaoui, S. (2005) Ecological quality of watercourses in eastern Algeria: case of the Oued Rhumel in the Constantinois region. Deuxième Congrès Méditerranéen: Ressources en eau dans le Bassin Méditerranéen : WATMED 2. Marrakech, Maroc. 14-17 Novembre, Proceeding CD-Rom. 1-10. (In French).

[4] Achour, S., Modjad, H., Hellal, H., Kelili, H. (2019) Trials to optimize clarification and desinfection processes for dam water from the Khenchela region (eastern Algeria). Larhyss Journal. 37, 151-174. (In French).

[5] Smatti-Hamza, I., Afri-Mehennaoui, F.Z., Keddari, D., Mehennaoui, S. (2019) Level of contamination by the metallic trace elements cadmium, cobalt, copper and zinc of two cyprinids and sediments from the Koudiet Medouar dam (Batna, Algeria).Journal of Applied Biosciences. 143, 14606–14621. (In French).

[6] Savary, P. (2010) Guide to water quality analysis. Groupe Territorial, France. Pp266. (In French).

[7] Boulaksaa, K., Laifa, A. (2020) Evaluation of the mineral nitrogen pollution of the surface waters of the Ramsar wetland of Lake Fetzara (North-East Algeria). Revue des Sciences de l'Eau / Journal of Water Science. 32(4), 409–419.

[8] Bennamoun, S., Boumazbar, M. (2018) Contribution to the study of the physico-chemical quality of the waters of the Koudiet M'daour dam (Wilaya of Batna). Mémoire de Master, Université des Fréres Mentouri Constantine. Pp: 28. (In French).

[9] Debbih, H., Naili, B.E. (2015) Study of water quality in dams in Eastern Algeria. Mémoire de Master, Université Oum El Bouaghi. Pp: 67. (In French).

[10] Rodier, J., Bazin, C, Broutin, J, Champsaur, H., Rodi, L. (2005) Water analysis. Naturel water. Wastewater. Sea water. 8th Ed. Dunod. Paris. Pp1383. (In French).

[11] Mouaissia, W. (2017) Contribution to the study of the biology (growth, morphometric structure, reproduction and diet) of the fish species *Barbus callensis* (Cyprinids) in the Beni Haroun (eastern Algéria). Thèse de doctorat, Université Mohamed Cherif Messadia, Souk Ahras. Pp140. (In French).

[12] Montuelle, B. (2003) Freshwater sediment Quality and management. physico-chemical and biological elements. Cemagref, Lyon, France. Pp291. (In French).

[13] Merabet, S. (2010) Assessement of the physico-chemical quality of raw and distributed water from the Beni Haroun reservoir dam. Mémoire de magister chimie analytique, Université des Fréres Mentouri, Constantine. Pp 4, 5+9. (In French).

[14] Loup, J.P. (1974) Terrestrial waters. Collection dirigée par Jean Pelletier. Masson et Cie. Paris Ive 1974. 8-14. (In French).

[15] Eckenfelder, W. (1982) Urban and industrial wastewater management. Technical documentation. Ed. Lavoisier, Paris. Pp503. (In French).

[16] Allalgua, A., Kaouachi, N., Boualeg, C., Ayari, A., Bensouileh, M. (2017) physico-chemical characterization of water from Foum El-Khanga dam (Souk-Ahras region, Algeria). European Scienbtific Journal (ESJ). 13(12), 258-275. (In French).

[17] Lhadi, E.K., Mountadar, M., Tounsi, A. (1996) Nitrate Pollution of groundwater in the coastal zone of d'El Jadida (Morocco). Hydrogeol. 3, 21-23. (In French).

[18] Merghem, K.A., Elhalouani, H., Alnedhary, A.A., Dssouli, K., Gharibi, E., Alansi, R.Q., Alnahmi, F. (2016) Study of the impact of raw and treated wastewater discharges on the quality of Oued Bani Houat (Sanaa bassin) : Spatio-temporal study. J. Mater. Environ. Sci. 7, 1516-1530. (In French).

[19] Journal Officiel de la République Algérienne (JORA) (2011) International conventions and agreements. Laws and decrees, decisions, notices, communications and announcements. JORA. 13, 1-24. (In French).

[20] Harrat, N., Achour, S. (2010) Physical and chemical pollution of dam water in the El Tarf region. Impact sur la chloration. Larhyss Journal. 8, 47-54. (In French).
[21] Ben Moussa, A., Chahlaoui, A., Rour, E.H., Chahboune, M., Aboulkacem, A. (2012) Study of the change in the state of the Oued Khoumane waters at the confluence with the thermal waters of the Ain Hamma Moulay Idriss Morocco. Larhyss Journal. 11, 17-36. (In French).
[22] Derwich, E., Beziane, Z., Benaabidate, L., Belghyti, D. (2008) Assessment of surface water quality in Fès and Sebou wadis used for market gardening in Morocco. Larhyss Journal. 07, 59-77. (In French).
[23] Mehanned, S., Chahlaoui, A., Zaid, A., Samih, M., Chahboune, M. (2014) Typology of the physico-chemical quality of water from Sidi Chahed dam in Morocco. J. Mater. Environ. Sci. 5(5), 1633-1642. (In French).
[24] Derwich, E., Benaabidate, L., Zian, A., Sadki, O., Belghity, D. (2010) Physico-chemical characterization of the alluvial groundwater of the Haut Sebou downstream of its confluence with Oued Fes. Larhyss Journal. 08, 101-112. (In French).
[25] Menasria, A. (2021) Eco-biology and Helminth parasites of *Barbus* species in some water reservoirs ineastern Algeria. Thèse de doctorat, Université Mohamed Cherif Messadia. Souk Ahras. Pp 215. (In French).
[26] Melghit, M. (2009) Physico-chemical quality, organic and metallic pollution of water / sediments compartments of Oued Rhumel, Hammam Grouz and Beni Haroun dams. Thèse de Magister en Ecologie, Université des Fréres Mentouri. Constantine. Algérie. Pp132. (In French).

**Received:** **31.07.2023**
**Accepted:** **11.08.2023**

## CORRESPONDING AUTHOR

**Menasria Amel**
Laboratory of Terrestrial and Aquatic Ecosystems, Faculty of Natural and Life Sciences,
University of Mohamed Cherif Messaadia, BP 1553, Annaba Road, 41000 Souk Ahras – Algeria.

e-mail: a.menasria@univ-soukahras.dz

-© by PSP Volume 32– No. 09/2023 pages 2988-2996 Fresenius Environmental Bulletin

# IMPACT OF THE FREQUENCY OF SORTED WASTE COLLECTION ON THE SORTING RATE OF MIXED MUNICIPAL WASTE IN SLOVAKIA

**Ivan Il'ko[1,*], Viera Peterkova[2]**

[1,2]Department of Biology, Faculty of Education, Trnava University in Trnava, Trnava, Slovak Republic

## ABSTRACT

In this paper we look at the impact of the frequency of waste collection in Slovak cities and municipalities and its impact on the sorting rate of mixed municipal waste. We focused on the occurrence of collection bins for sorted waste (paper, plastic, glass, metal) and the frequency of their export in 22 municipalities and towns in Slovakia. By analysing a sample of mixed municipal waste (11891.6 kg), we found the average percentage share of each waste component in mixed municipal waste in each municipality, and we further looked only at the 4 waste components mentioned above (paper 7.02%; plastic 11.32%; glass 5.07%; metal 2.74%). We demonstrated a statistically significantly lower percentage of paper (p= 0.0124) in mixed municipal waste in municipalities where the frequency of paper export was on a weekly basis, compared to monthly paper export. Similar results were obtained for glass (p= 0.0267) and metal (p= 0.0090). We did not show a statistically significantly lower percentage of plastic (p= 0.7904). We further looked at the comparison of urban (over 5,000 inhabitants) and rural (under 5,000 inhabitants) and individual housing, and complex housing, and did not show a statistically significant difference in any of the waste components studied. We can conclude that an increase in the frequency of export of sorted collection may have a positive impact on the rate of separation of mixed municipal waste in Slovakia. Other measures leading to higher sorting rates are, for example, education of the population, lower delivery distances, availability of containers for sorted waste, etc.

**KEYWORDS:**
Frequency of waste export, mixed municipal waste, analysis, sorted waste

## INTRODUCTION

Waste is a product of human society and its activities arise in all spheres of material production. Not only does it release greenhouse gases into the atmosphere, but its improper management hampers efforts to keep places beautiful and clean. Improperly treated municipal solid waste pollutes soil, water, and the atmosphere (releasing greenhouse gases such as methane, nitrous oxide, and carbon dioxide), which can also have adverse effects on public health [1]. The authors define waste as an unnecessary product or substance that we do not want or for certain reasons cannot be used anymore [2]. Waste threatens all biotic and abiotic components of the environment (e.g. water, soil or air) [3]. First step in dealing with waste is to prevent its generation [4]. Next comes reuse, recycling and composting, incineration (i.e. its energy recovery) and lastly landfilling. Municipal waste is the waste that is produced by households and businesses. This group also includes bulky waste, urban green waste, and street waste if it has a similar composition to household waste. Household waste is composed of several components, including organic waste, glass, paper, plastics, textiles, metals, packaging materials and mixed municipal waste. Mixed municipal waste is the part of municipal waste that has not undergone the process of re-sorting [5]. All the waste generated needs to be handled in an appropriate manner so that the environment is minimally polluted. Waste handling is a concept that expresses the operations that start at the place where the waste was generated and end at the place of treatment or disposal of the waste. These operations consist of the collection, assembly and removal of waste. The place where the waste is generated is the area where material handling activity generates waste. It can be a household, a health care facility or even an industry. Due to the exponential increase in the volume of municipal solid waste due to rapid population growth in urban areas, municipal solid waste management has become a major challenge in urban planning [6].

With the increasing environmental literacy of people and the worsening of global environmental problems, there is a growing demand for sustainable and conscious waste management and disposal [7]. There are laws, ordinances and selected regulations in the territory of the Slovak Republic in the field of waste and its disposal. Since the Slovak Republic is a member state of the European Union, it has to comply with the legislation issued and in force throughout the European Union. Act No 223/2001 Coll. on waste includes waste management programmes, the

competence of state waste management authorities, the obligations of legal and natural persons in waste management, transboundary shipments of waste, import of waste, export and transit of waste, waste management, and contributions to the recycling fund.

According to the Waste Management Programme of the Slovak Republic for the years 2021 - 2025, the main objective of the waste management by 2025 is to divert waste away from disposal by landfilling, especially for municipal waste. It is necessary to continue to promote compliance with the waste hierarchy, with an emphasis on waste prevention, preparation for re-using and recycling. The promotion of waste prevention, together with reuse and preparation for reuse, including through the implementation of the measures of the Waste Prevention Programme for 2019-2025, are an integral key part of the long-term efforts of the Slovak Republic to reduce the amount of waste generated on the territory of the Slovak Republic [8]. According to Act No. 223/2001 Coll. on waste, the municipality is the originator of municipal waste generated on its territory. Therefore, each municipality must draw up its Waste Management Programme [9].

The authors state that households play a key role in reducing the environmental impact of municipal waste, which they influence both by reducing production and also by sorting recoverable waste components [10]. Understanding their behaviour and identifying the factors that influence them is essential to set up and use municipal or state tools in a way that will lead to the desired results. The authors have shown that culture has a significant influence on peoples' behaviour even in the context of waste [11]. The authors argue that an individual's tendencies to reduce waste are unrelated to their tendencies to sort waste, i.e., individuals who reduce waste are not automatically expected to sort waste, and vice versa [12]. They further note that similar reasons may drive people to sort and to reduce waste. Factors influencing the level of sorting of municipal waste can be divided into three categories - external factors (demographic and socio-economic factors), internal factors (socio-psychological factors), and system characteristics [13]. Factors influencing waste reduction and reuse coincide with those influencing the level of sorting. They are mainly environmental values, general knowledge of the issue, perception of waste as a problem threatening human well-being, convenience of the system, and belief in the effectiveness of one's actions [14]. Internal factors can be worked with to some extent by the municipality, especially the factor of legal norms, information, and knowledge. The municipality can establish a certain behaviour as a legal norm through its normative instrument of a municipally binding ordinance. The municipality also has information tools at its disposal through which it can raise awareness of the issue [15]. The municipality has the greatest influence on the characteristics of the system as it is the creator of the waste management system. According to authors, it is the characteristics of the system that is the category of factors that influences household behaviour the most [13]. The most important factors are the subjective perception of the convenience of the system, which essentially encompasses all the characteristics of the system (e.g. availability of collection bins, frequency of waste collection and export, etc.) [16-17]. Another factor is how the system is charged [13].

The aim of this study was to investigate the impact of the frequency of waste collection in Slovak municipalities on the sorting rate of mixed municipal waste. We focused on the occurrence of collection bins for sorted waste (paper, plastic, glass, metal) and the frequency of their export in 22 municipalities and towns in Slovakia. This study can contribute in the government's efforts to increase the waste sorting rate in Slovakia, as an effective waste sorting rate is an essential attribute for smart cities. The contribution of this study has several levels. We address one of the main drivers of waste sorting rates, considering the impact of sorting rates for different waste components, thereby identifying waste components that may not be subject to the trend. Insufficient frequency of waste collection can lead to accumulation of waste outside collection bins, the creation of illegal landfills, and also an increase in the growth of mixed municipal waste. Next, we look at the difference between towns and villages, and individual and complex housing developments. The data provided and processed by us can be used by cities in setting the appropriate frequency of sorted collection exports as well as in designing solid waste management plans.

## MATERIALS AND METHODS

Our analysis of mixed municipal waste was based on the legislative guidance of the Ministry of the Environment of the Slovak Republic, which according to § 105 (3) (a) of Act No. 79/2015 Coll. on Waste and on Amendments and Additions to Certain Acts provides in § 1 Methodology of Mixed Waste Analysis [18]. This measure entered into force on 1st July 2020. Mixed waste analysis is a detailed examination of mixed waste in terms of its composition. The sample size of mixed waste shall be set at between 5 % and 10 % of the total number of collection containers for mixed waste in use in the area of interest. The bins shall not come from a single location in the area of interest, for example from a single street, street block, housing estate or urban area, the sample shall be from as large area of interest as possible, unless the aim of the analysis is to analyse the composition of mixed waste from only one location. In selecting the collection receptacles to be sampled, receptacles containing sub-standard waste or any of the waste

components in sub-standard quantities shall be excluded. Data on the weight of the separated waste fractions shall be entered in a table according to the individual waste fractions separated in kilograms. Our analyses were determined from 5% of the total number of collection bins for mixed waste in 22 municipalities in the Slovak Republic (Table 1). The present work focuses on the influence of the frequency of waste collection in Slovak cities and municipalities on the sorting rate of mixed municipal waste. We focused on the occurrence of collection bins for sorted waste (paper, plastic, glass, metal) and the frequency of their export. By analysing a sample of mixed municipal waste from 22 towns and municipalities in Slovakia, we obtained 11891.6 kg of waste. Furthermore, we determined the average percentage of each waste component in the mixed municipal waste in each municipality, and we further looked only at: paper 7.02%; plastic 11.32%; glass 5.07% and metal 2.74%. We further looked at the comparison of city (over 5,000 inhabitants) and village (up to 5,000 inhabitants) and individual housing construction (IHC) and complex housing construction (CHC) (Act No. 369/1990 Coll.) [19].

**TABLE 1**
**Total waste and shares of plastic, paper, glass, and metal in 22 study areas**

| Municipality | IHC/CHC | Town/ village | Waste in total | Share of paper | Share of plastic | Share of glass | Share of metal |
|---|---|---|---|---|---|---|---|
| **Pezinok** | IHC | T | 223.2 | 8.47 | 10.22 | 4.03 | 2.28 |
| **Pezinok** | CHC | T | 138.5 | 9.46 | 9.75 | 2.89 | 2.24 |
| **Rakovice** | IHC | V | 221.5 | 5.42 | 9.48 | 2.03 | 4.06 |
| **Rakovice** | IHC | V | 143 | 4.55 | 12.24 | 1.75 | 3.15 |
| **Hontianska Vrbica** | IHC | V | 147.8 | 3.99 | 9.27 | 4.26 | 3.38 |
| **Veľký Ďur** | CHC | V | 39 | 8.97 | 12.82 | 5.13 | 2.56 |
| **Veľký Ďur** | IHC | V | 523.5 | 1.53 | 7.64 | 2.48 | 4.68 |
| **Vráble** | IHC | T | 648.8 | 11.99 | 11.25 | 4.96 | 1.59 |
| **Topoľčany** | CHC | T | 1,166.87 | 5.85 | 8.82 | 4.57 | 1.77 |
| **Topoľčany** | IHC | T | 174.31 | 9.15 | 9.69 | 4.88 | 4.22 |
| **Krásno nad Kysucou** | IHC | T | 374 | 5.35 | 8.82 | 8.02 | 2.67 |
| **Bystrička** | IHC | V | 179.39 | 9.35 | 6.82 | 7.11 | 1.42 |
| **Poniky** | IHC | V | 182.33 | 6.17 | 11.24 | 6.25 | 4.66 |
| **Slovenská Lupča** | IHC | V | 263.68 | 6.51 | 4.48 | 3.81 | 2.40 |
| **Slovenská Lupča** | CHC | V | 183.7 | 3.09 | 5.61 | 3.18 | 5.36 |
| **Valaská** | CHC | V | 201.77 | 2.17 | 16.16 | 4.66 | 2.18 |
| **Valaská** | IHC | V | 40.8 | 2.45 | 21.32 | 2.45 | 2.45 |
| **Petrovce** | IHC | V | 87.17 | 7.45 | 9.01 | 5.22 | 2.36 |
| **Orovnica** | IHC | V | 167.86 | 16.28 | 12.33 | 5.54 | 1.85 |
| **Orovnica** | CHC | V | 80.23 | 11.22 | 14.53 | 3.74 | 1.74 |
| **Horná Ves** | IHC | V | 242.3 | 6.48 | 13.37 | 5.16 | 3.63 |
| **Ľubica** | IHC | V | 218.86 | 3.82 | 13.41 | 2.28 | 1.37 |
| **Spišský štvrtok** | IHC | V | 104.3 | 12.13 | 4.79 | 4.60 | 2.73 |
| **Dlhé nad Cirochou** | IHC | V | 324.79 | 3.74 | 11.60 | 7.11 | 3.88 |
| **Dlhé nad Cirochou** | IHC | V | 254.88 | 12.20 | 15.11 | 8.95 | 2.39 |
| **Snina** | IHC | T | 682.3 | 6.39 | 13.19 | 5.25 | 1.88 |
| **Snina** | CHC | T | 565.6 | 9.79 | 13.07 | 6.42 | 1.15 |
| **Vranov nad Topľou** | IHC | T | 984.78 | 5.98 | 6.92 | 6.42 | 1.14 |
| **Vranov nad Topľou** | CHC | T | 463.7 | 9.88 | 12.66 | 7.76 | 2.26 |
| **Prakovce** | IHC | V | 682 | 5.59 | 14.21 | 11.33 | 3.20 |
| **Trebišov** | IHC | T | 279.5 | 4.83 | 9.30 | 6.08 | 1.18 |
| **Trebišov** | CHC | T | 629.8 | 9.54 | 11.45 | 5.46 | 2.29 |
| **Sečovce** | IHC | T | 372.38 | 2.97 | 18.28 | 3.20 | 4.51 |
| **Sečovce** | CHC | T | 827 | 5.83 | 16.06 | 5.34 | 4.11 |

## RESULTS

The data collected were recorded in a spreadsheet (Microsoft Excel), which serves as the basis for this work. Statistically processed data were subjected to analysis in Statistica, version 12. The analysis of the statistical file was based on the percentage composition of the waste. We used the Shapiro-Wilk test to determine normality. We further analyzed the statistical set using Kruskal-Wallis analysis of variance. We demonstrated a statistically significant lower percentage of paper ($p = 0.0124$) in mixed municipal waste in municipalities where the frequency of paper export was on a weekly basis, compared to monthly paper export (Figure 1).

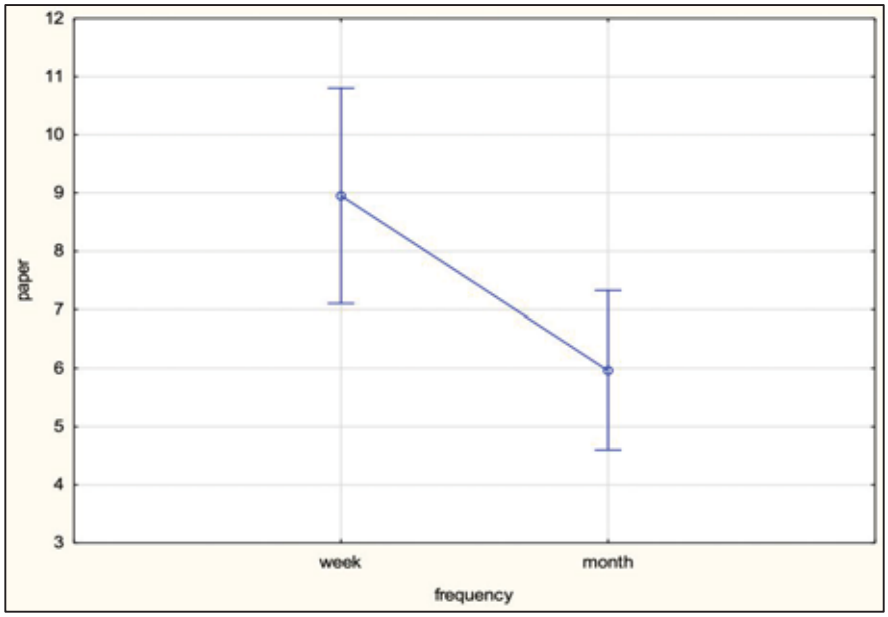



**FIGURE 1**
**Comparison of municipalities with weekly paper export frequency and monthly paper export frequency**

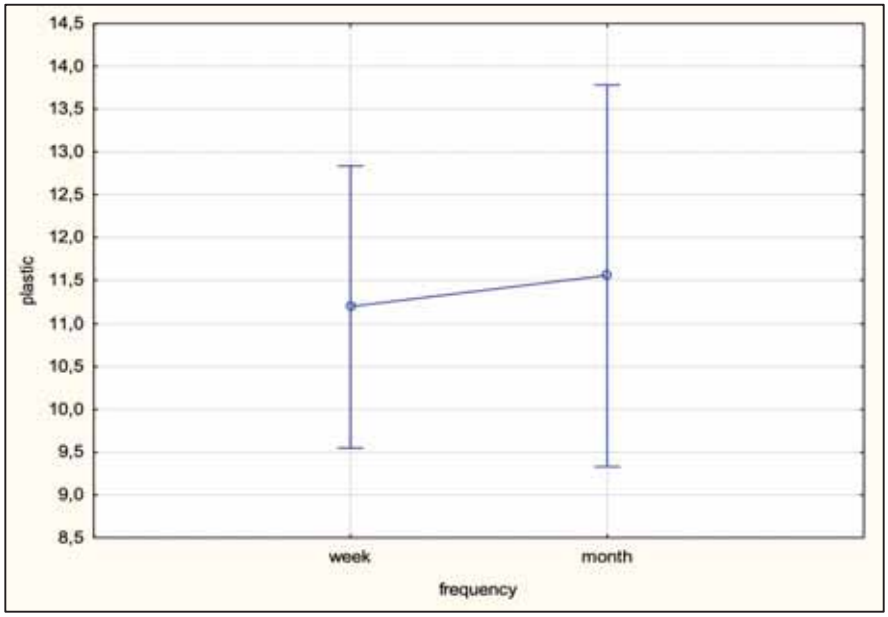



**FIGURE 2**
**Comparison of municipalities with weekly plastic export frequency and monthly plastic export frequency**

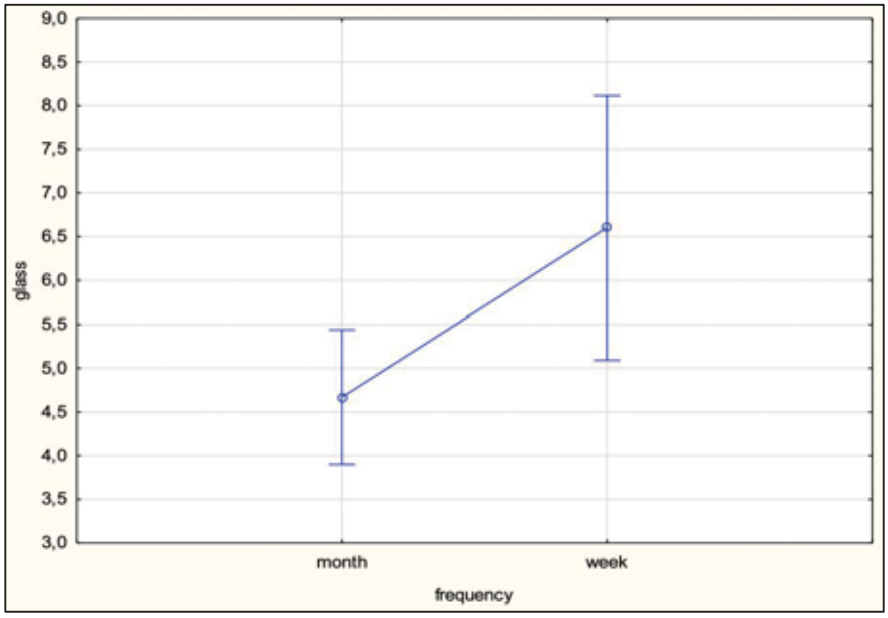



**FIGURE 3**
**Comparison of municipalities with weekly glass export frequency and monthly glass export frequency**

Further, we did not show a statistically significant lower percentage of plastic (p= 0.7904) in mixed municipal waste in municipalities where the frequency of plastic export was on a weekly basis, compared to monthly plastic export (Figure 2).

We demonstrated a statistically significant lower percentage of glass (p= 0.0267) in mixed municipal waste in municipalities where the frequency of glass export was on a weekly basis, compared to monthly glass export (Figure 3).

We demonstrated a statistically significant lower percentage of metal (p= 0.0090) in mixed municipal waste in municipalities where the frequency of metal export was on a weekly basis, compared to monthly metal export (Figure 4).

Next, we looked at the difference between individual housing developments and complex housing developments and town and village. We did not find a statistically significant difference between town and village in the frequency of waste export and its percentage in mixed municipal waste in any of the waste components studied: paper (p= 0.4290), plastic (p= 0.2300), glass (p= 0.3587) and metal (p= 0.1961) (Figure 5).

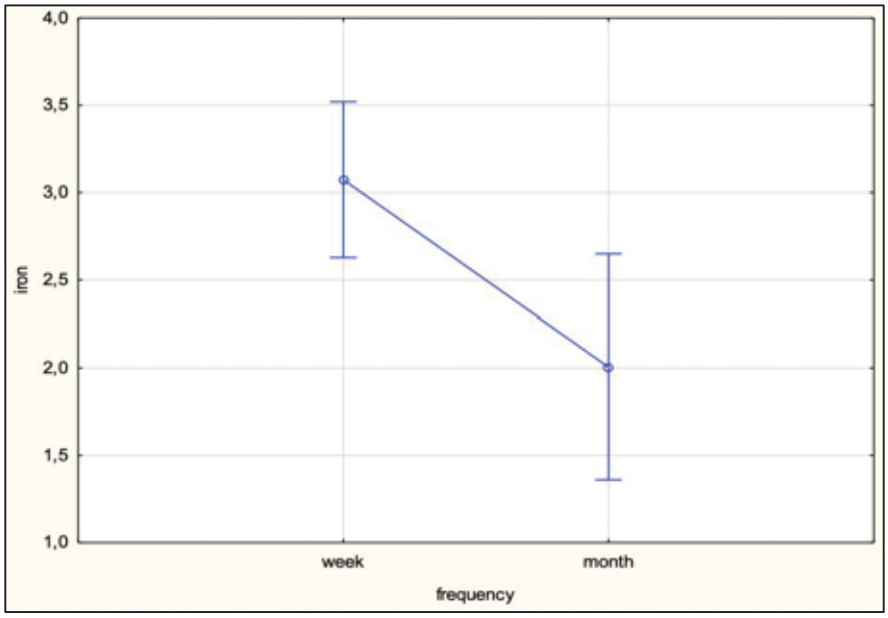



**FIGURE 4**
**Comparison of municipalities with weekly metal export frequency and monthly metal export frequency**

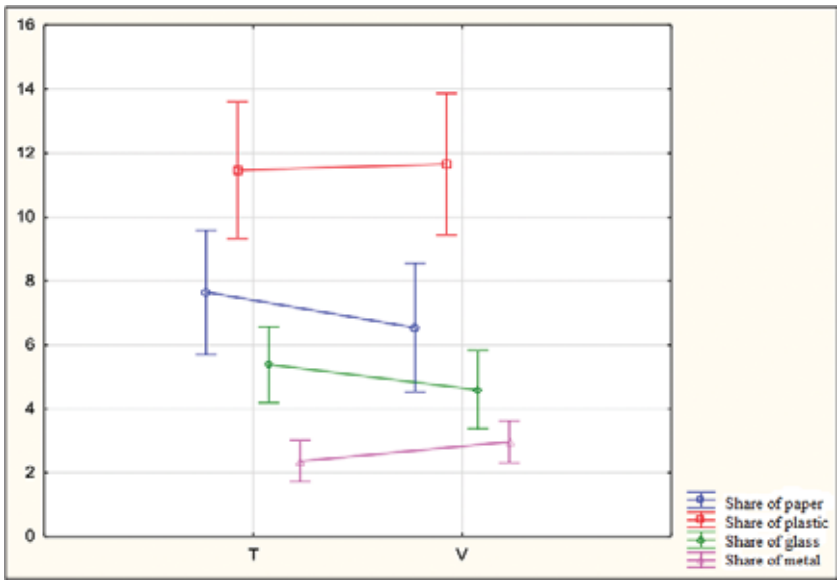



**FIGURE 5**
**Difference between town and village in the frequency of waste export and its percentage in mixed municipal waste**

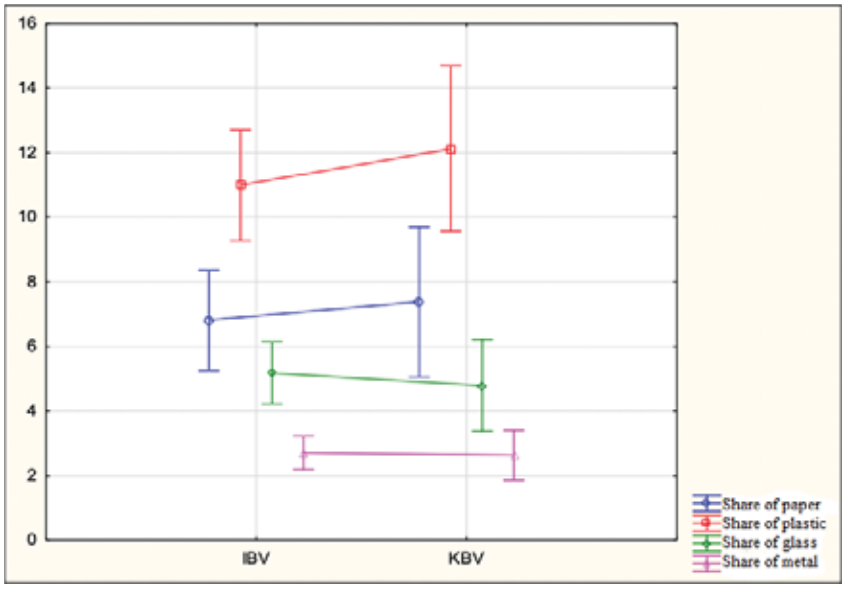



**FIGURE 6**
**Difference between individual residential construction and complex residential construction in the frequency of waste export and its percentage share in mixed municipal waste**

Similarly, we did not find a statistically significant difference between individual housing developments and complex housing developments in the frequency of waste export and its percentage share in mixed municipal waste in any of the waste components studied: paper (p= 0.6812), plastic (p= 0.4571), glass (p= 0.6404) and metal (p= 0.8731) (Figure 6).

## DISCUSSION

Efficient waste management is the basis for improving services to citizens [20-21]. Therefore, this study attempted to understand the effect of frequency of sorted waste collection on the sorting rate of mixed municipal waste. In our study, we estimated that weekly waste collection frequency has a statistically significant effect on the sorting rate of mixed municipal waste compared to monthly sorted waste collection frequency. We showed statistical significance in several waste components paper, metal and glass, while for the plastic component of mixed waste we did not show a statistically significant difference between weekly and monthly collection frequency. Also authors showed that plastic (16.8%) is the most represented component (among the waste components we observed) in the mixed municipal waste [22]. Further, we did not show any

statistically significant difference when comparing town and village and individual housing development and complex housing development.

In addition to the frequency of waste collection, many other factors influence the rate of waste sorting and production. Waste generation rates may increase with increasing income [23]. The authors state that demographic factors have only a negligible impact on Czech households [24]. Conversely, for example, other research has shown differences in municipal waste production and separation between different age groups [25]. Technical conditions in the household, such as sufficient space for sorting in the kitchen, is an important factor [26]. It is also important to inform and reassure citizens that their efforts are worthwhile and that the waste they sort is actually used for its declared purpose and does not end up in a landfill or incinerator [27]. Social norms can be understood, for example, as the dominant pattern of behaviour in a particular group [28]. The authors suggest that the social pressure on an individual's willingness to sort waste is based on a sense of belonging or being part of a community [13]. Authors also point to a social norm factor in the system of collecting separated components directly from households, where the presence of multiple bins at the dwelling represents a means of mutual social control [29]. It serves as visible evidence to people in the nearby community that the individual is behaving in accordance with the socially accepted norm, and thus becomes an incentive factor for sorting. This study indicates that the provision of collection nests or the collection of segregated waste components directly from households has a positive impact on waste sorting rates, as it represents saved time and space for people otherwise required to store individual components and to take this waste to more distant collection yards [30]. The introduction of door-to-door collections then has an even more significant impact on sorting rates than the provision of collection nests. The same conclusion was reached in a study in which the authors claim that a door-to-door collection system increases the level of waste separation by up to 40% compared to a nested collection system [31]. The impact of the charging method was studied in 2003 and authors showed that municipal waste generation is lower in municipalities that have some form of charging than in municipalities that have a flat fee [26]. Incentive payments can double the level of waste sorting [31]. In this study authors found through research in Sweden that financial incentives influence households but are not the only motivation behind waste reduction and sorting [32]. If the infrastructure is available and there is a sufficient frequency of export of collection bins to allow easy separation, people are willing to invest more effort and time in these activities. The authors go on to state that the provision of separate collection of a large range of material waste components will not in itself lead to increased sorting rates unless it is also accompanied by a sufficient information campaign to ensure that citizens understand how to participate in the system properly. The authors make the same point by stating that the introduction of a better waste collection system is only one part of the problem [33].

The public must be informed to treat waste as a resource and use the system as such. Awareness of how to sort waste properly has also been shown to be an important factor [26]. The research shows that providing information about the environmental impacts of waste production increases the level of awareness of the issue, which in turn increases peoples' belief in the effectiveness of their actions, i.e. the belief that their actions contribute to solving the problem [34].

The issue of waste can be considered as one of the major environmental problems of our time. A number of factors contribute to an efficient, long-lasting and convenient waste sorting system. The findings presented by us point more deeply to the frequency of waste collection. On the other hand, it is not correct to view this factor in isolation. The individual factors we describe in this paper are inherent in the overall waste management setup in a city or village and subsequently in the state. For effective waste collection and segregation rates, it is necessary to take into account all the factors that influence the rate of waste segregation and production. By effectively introducing the different factors into waste policies, we can increase the waste sorting rate and reduce the production of mixed municipal waste in the Slovak Republic.

## CONCLUSION

In our study, we look at how the sorting rate of mixed municipal waste can be improved by increasing the frequency of sorted collection exports. In order to handle sorted waste effectively, it is first of all, important to increase the sorting rate and to focus on reducing the production of mixed waste. Like other cities, municipalities in Slovakia face a number of challenges in solid waste management. It is important for municipalities to focus on the participation of all households in municipal solid waste management, while addressing complaints from households already participating in the municipal solid waste management program, so as not to reduce their involvement and act as a motivating factor for non-participating residents. The results of our work suggest that the increase in municipal mixed waste can be curbed by increasing the frequency of export of sorted collection. Residents reduce sorting rates when sorted waste bins are full, while creating illegal dumping sites or placing waste outside the bins, which complicates waste collection, looks unaesthetic and can promote the spread of odours, which can attract rodents and insects. The trend we have

shown has not been demonstrated for salaries, which may be related to peoples' reluctance to sort plastic waste. Introducing a deposit system for all plastic packaging material and educating people may help to address the situation. We also see solutions in increasing municipal waste charges or introducing door-to-door collection of sorted waste. Further study is needed to investigate incentives that would motivate residents and waste generators to increase waste sorting rates. This could be an effective way of building a sustainable waste management system in Slovakia.

## ACKNOWLEDGEMENTS

The authors would like to thank JRK Slovensko s.r.o. for their cooperation in carrying out the analyses of municipal waste and providing data for research processing.

## REFERENCES

[1] Ferronato, N. and Torretta, V. (2019) Waste Mismanagement in Developing Countries: A Review of Global Issues. International Journal of Environmental Research and Public Health. 16(6), 1060.

[2] Vybíralová, J., Füzyová, Ľ., Polačko, V. (2005) Creation and protection of the environment. EKONÓM. 234. ISBN 80-225-2098- 5 (in Slovak).

[3] Chmielewská, E. (2011) Protection and use of natural resources. Epos, Bratislava, 349. ISBN: 978-80-8057-846-6 (in Slovak).

[4] Bočková, V. (2004) Slovak Environmental Agency. Hierarchy of waste management. Banská Bystrica. Available online: <http://enviroportal.sk/environmentalne-temy/odpady>. [cit. 3.7.2023] (in Slovak).

[5] Rimárová, K. (2008) Selected chapters from hygiene - environmental medicine. Elfa s.r.o. 251. ISBN 978-80-8086-090-5 (in Slovak).

[6] Kumar, R., Bhattarai, D. and Neupane, S. (2019) Designing solid waste collection strategy in small municipalities of developing countries using choice experiment. Journal of Urban Management. 8(3), 386–395.

[7] Sujauddin, M., Huda, S.M.S. and Hoque, A.T.M.R. (2008) Household solid waste characteristics and management in Chittagong, Bangladesh. Waste Management. 28(9), 1688–1695.

[8] Author unknown. (2021) Waste Management Programme of the Slovak Republic for the years 2021 – 2025. Available online : https://www.enviroportal.sk/odpady/program-odpadoveho-hospodarstva-slovenskej-republiky-2021-2025. (Accessed Date:24.11.2021) (in Slovak).

[9] National Council of the Slovak Republic. (2001). Act No. 223/2001 Coll., the Waste Act and the amendment and supplementation of certain acts. https://www.slov-lex.sk/pravne-predpisy/SK/ZZ/2001/223/20150101.html .(Accessed Date: 2023-6-6) (in Slovak).

[10] Van Der Werff, E., Vrieling, L., Van Zuijlen, B. and Worrell, E. (2019) Waste minimization by households – A unique informational strategy in the Netherlands. Resources Conservation and Recycling. 144, 256–266.

[11] Mintz, K.K., Henn, L., Park, J. and Kurman, J. (2019) What predicts household waste management behaviors? Culture and type of behavior as moderators. Resources Conservation and Recycling. 145, 11–18.

[12] Ebreo, A. and Vining, J. (2001) How Similar are Recycling and Waste Reduction? Environment and Behavior 33(3), 424–448.

[13] Slavík, J., Lukáč, D., Hradilová, L. and Zákravská, R. (2018) Factors influencing citizens' motivation to sort-selected results for the Czech Republic. Waste and municipalities 2018, 115-118. Available online : https://www.ekokom.cz/uploads/attachments/OD/SBORN%C3%8DK%2018.pdf. (Accessed Date: 2023-7-23 ) (in Slovak).

[14] Barr, S. (2007) Factors Influencing Environmental Attitudes and Behaviors. Environment and Behavior. 39(4), 435–473.

[15] Tonglet, M., Phillips, P.S. and Bates, M.P. (2004) Determining the drivers for householder pro-environmental behaviour: waste minimisation compared to recycling. Resources Conservation and Recycling. 42(1), 27–48.

[16] Miafodzyeva, S. and Brandt, N. (2012) Recycling Behaviour Among Householders: Synthesizing Determinants Via a Meta-analysis. Waste and Biomass Valorization. 4(2), 221–235.

[17] Saphores, J. and Nixon, H. (2014) How effective are current household recycling policies? Results from a national survey of U.S. households. Resources Conservation and Recycling. 92, 1–10.

[18] National Council of the Slovak Republic (2015). Act No. 79/ 2015 Coll. on Waste and on Amendments and Additions to Certain Acts. https://www.zakonypreludi.sk/zz/2015-79. (Accessed Date: 2023-6-6) (in Slovak).

[19] National Council of the Slovak Republic. (1990). Act No. 369/1990 Coll. Act of the Slovak National Council on Municipal Establishment. https://www.zakonypreludi.sk/zz/1990-369. (Accessed Date: 2023-6-6) (in Slovak).

[20] Baud, I., Grafakos, S., Hordijk, M. and Post, J. (2001) Quality of Life and Alliances in Solid Waste Management. Cities. 18(1), 3–12.

[21] Lytras, M.D. and Visvizi, A. (2018) Who Uses Smart City Services and What to Make of It: Toward Interdisciplinary Smart Cities Research. Sustainability. 10(6), 1998.
[22] Shehu, S. (2017) Separation of plastic waste from mixed waste: Existing and emerging sorting technologies performance and possibilities of increased recycling rate with Finland as case study. Lappeenranta University of Technology, Faculty of Technology, Lappeenranta.
[23] Pokhrel, D. and Viraraghavan, T. (2005) Municipal solid waste management in Nepal: practices and challenges. Waste Management. 25(5), 555–562.
[24] Rybová, K., Slavík, J., Burcin, B., Soukopová, J., Kučera, T. and Černíková, A. (2018) Socio-demographic determinants of municipal waste generation: case study of the Czech Republic. Journal of Material Cycles and Waste Management. 20(3), 1884–1891.
[25] Struk, M. (2017). Distance and incentives matter: The separation of recyclable municipal waste. Resources Conservation and Recycling. 122, 155–162.
[26] Sauer, P. (2003) How (not) to pay for household waste: contributions of the final seminar of the project PAYT. Oeconomica. 176. ISBN 80-245-0638-6 (in Slovak).
[27] Ribić, B., Pezo, L. and Ilakovac, B. (2016) Concept of sustainable waste management in the city of Zagreb: Towards the implementation of circular economy approach. Journal of the Air & Waste Management Association. 67(2), 241–259.
[28] Nyborg, K., Anderies, J.M., Dannenberg, A., Lindahl, T., Schill, C., Schlüter, M., Adger, W.N., Arrow, K.J., Barrett, S., Carpenter, S.R., Chapin, F.S., Crépin, A., Daily, G.C., Ehrlich, P.R., Folke, C., Jager, W., Kautsky, N., Levin, S.A., Madsen, O., De Zeeuw, A. (2016) Social norms as solutions. Science. 354(6308), 42–43.
[29] Barr, S., Gilg, A.W. and Ford, N. (2001) A Conceptual Framework for Understanding and Analysing Attitudes towards Household-Waste Management. Environment and Planning A. 33(11), 2025–2048.
[30] Jenkins, R.R., Martinez, S., Palmer, K. and Podolsky, M.J. (2003) The determinants of household recycling: a material-specific analysis of recycling program features and unit pricing. Journal of Environmental Economics and Management. 45(2), 294–318.
[31] Struk, M. and Soukopová, J. (2016) Age structure and municipal waste generation and recycling–new challenge for the circular economy. In 4th International Conference on Sustainable Solid Waste Management, 23rd-25th June 2016 Limassol, Cyprus. 5-6.
[32] Bartelings, H. and Sterner, T. (1999) Household waste management in a Swedish municipality: determinants of waste disposal, recycling and composting. Environmental and Resource Economics. 13(4), 473–491.
[33] Williams, I.H. and Taylor, C.B. (2004) Maximising household waste recycling at civic amenity sites in Lancashire, England. Waste Management. 24(9), 861–874.
[34] Van Der Werff, E., Vrieling, L., Van Zuijlen, B. and Worrell, E. (2019 b) Waste minimization by households – A unique informational strategy in the Netherlands. Resources Conservation and Recycling. 144, 256–266.

**Received:** **01.08.2023**
**Accepted:** **14.08.2023**

**CORRESPONDING AUTHOR**

**Ivan Il'ko**
Department of Biology
Trnava University in Trnava
Trnava – Slovak Republic

e-mail: ivan.ilko@truni.sk

-© by PSP Volume 32– No. 09/2023 pages 2997-3006 Fresenius Environmental Bulletin

# SOIL ORGANIC CARBON STORAGE UNDER CITRUS: PRELIMINARY STUDY IN THE COASTAL ZONE OF NORTHWESTERN ALGERIA

**Benouadah Salima**[1,2,*]

[1]Laboratory of Biodiversity, Water and Soil Conservation, Faculty of Natural and the Life Sciences, Abdelhamid Ibn Badis University, BP 188/227 Mostaganem, 27000, Algeria
[2]Laboratory of Agro-Biotechnology and Nutrition in Semi-Arid Zones, Faculty of Natural and the Life Sciences, Ibn Khaldoun University, BP 78 Zaâroura 14000, Tiaret, Algeria

## ABSTRACT

Atmospheric carbon sequestration in terrestrial ecosystems has become an important issue in the global framework of reducing greenhouse gas emissions. The main objective of this study is to estimate the change in soil organic carbon stock (SCOS) of the agricultural workshop of Mazagran at Mostaganem. To do this, we quantified the organic carbon according to the main land uses represented by three different types of citrus crops: orange cultivation, grapefruit cultivation and lemon cultivation in comparison with spontaneous vegetation from the same study area to a depth of 30 cm. Stored organic carbon values varied significantly ($p<0.05$) between soil under citrus cultivation and soil under spontaneous vegetation, with the highest value recorded for soil under orange cultivation (51.26 ± 6.26 Mg C $ha^{-1}$), followed by soil under grapefruit cultivation (46.66 ± 4.69 Mg C $ha^{-1}$) and soil under lemon tree cultivation (42.76 ± 2.23 Mg C $ha^{-1}$ ), the lowest stock is recorded for soil under spontaneous vegetation (40.52 ± 4.48 Mg C $ha^{-1}$) , the average stock is 45.3 ± 11.45 Mg C $ha^{-1}$ for all 12 soil samples in the study area. More studies are necessary to comprehend the mechanisms of the formation of organic matter with several types of arboriculture, namely: citrus cultivation, viticulture, olive cultivation, etc., in relation to the length of the roots and according to several levels of soil depth such as: (0-15cm, 15-30cm, 30-45cm, 45-100cm).

**KEYWORDS:**
Organic carbon stocks, agricultural soils, citrus, land use, Mostaganem

## INTRODUCTION

Since the pre-industrial era (1880-1899), economic and demographic growth has led to an increase in the concentration of greenhouse gas emissions in the atmosphere, particularly carbonaceous gases ($CO_2$, $CH_4$) [1-2], this increase represents the main cause of the global warming [3-4].

From the period 1850-1900 to 2010-2019, the temperature of the earth's surface has increased at 1.07°C [4]. The temperature is not the only factor affected, the level of the oceans has risen, the glaciers have melted and extreme climatic events are more and more frequent [5]. The increase of greenhouse gases in the atmosphere by anthropogenic origin is responsible for all these changes, which will not if drastic measures are not put in place [6].

Soils play a key role in our environment through their ability to exchange nutrients and regulate [6]. Climate change is today the most important environmental crisis and soils can play a preponderant role essentially in regulation via the carbon cycle by storing organic carbon through photosynthesis in higher plants, which is the almost unique mode biological fixation of atmospheric $CO_2$ in the form of organic matter [7, 4].Carbon dioxide ($CO_2$) is the largest anthropogenic contributor to the greenhouse effect and climate change. In addition, approximately two-thirds of terrestrial organic carbon is found in the soil compartment [1]. In this context, soils seem to be a solution to mitigate climate change, as they represent the largest terrestrial reservoir of organic carbon [8-9].

The amount of soil organic carbon (SOC) is about twice as much as in the atmosphere and three times as much as in terrestrial vegetation, it is estimated at 1.5 trillion tons [10].

Soil organic matter (SOM), composed mainly of carbon, contributes to several ecosystem services for example to adapt to the effects of climate change: (i) water retention, precious during droughts, (ii) the richness nutrients, soil fertility and productivity, (iii) soil erosion resistance, (iv) increasing soil biodiversity [6], and (v) could play an essential role in reducing greenhouse gases emissions in the atmosphere and therefore in preventing of climate change.

International commitments to combat climate change have taken into account changes in the SOC in recent years [11].

According to the "4 by 1000" initiative, 4.3 billion tons of carbon are released into the atmosphere

each year [12], thus contributing to the greenhouse effect and global warming and that 1.2 billion tons of carbon could be stored in the agricultural topsoils per year, representing an annual storage rate of about 0.4%, through management practices adapted to local conditions. By soil carbon stabilization and destabilization mechanisms, these practices not influence carbon inputs only but also carbon outputs [4].

For semi-arid Mediterranean soils, carbon dynamics have received less attention than the other regions of the world [13]. Drylands contain about a third-one of the global stock due to the low productivity of the agro-ecosystems that they support and cover 40% of the land surface [14-15].

However, it is very clear that studies on the dynamics of organic carbon in agricultural soils are still insufficient, particularly in Algeria: [16] for a diachronic study (1988-2002) of Ouzéra soils, 90 km south of Algiers, [17] for another diachronic survey (1993-1998) in Beni-Chougrane, [18] for a comparative study of SOC on the Beni-Chougrane and Tlemcen mountains, [19] for another comparative study of the SOCS of Annaba and Sétif, in northeastern Algeria, [20] for a four soils study along a toposequence in the Zeramna valley at El Hadaiek, 5 km from Skikda in northeastern Algeria, [21] for the SOC estimation of forest soils in the Tessala Mountains of Sidi Bel Abbes, 440 km northwest of Algiers, [22] for the SOC improvement of Tiaret soil's under organic amendments, 320 km south-west of Algiers, [23] for the estimation of the SCO of agricultural soils in the plain of Sidi Bel Abbes.

According to [24, 22], the dynamics and the stabilization of organic matter are influenced significantly by land use and the land use system. The knowledge of the potential offered by agricultural soils is crucial according to land uses and practices [25].

This is why we contribute through this study to better understand the behavior of agricultural soils according to its occupation in semi-arid Mediterranean conditions in order to quantify the storage of organic carbon, taking into account the importance of the area occupied by the cultivation of citrus in Algeria, about 45,000 Hectares (11% of the surface occupied by fruit trees) and that it is one of the major Mediterranean citrus-producing countries, given that it has a varietal collection made up of 178 varieties of citrus fruits constituting an invaluable genetic heritage [26]. A quantity of 1.507 million quintals of different citrus fruits varieties was produced in the wilaya of Mostaganem during the 2021/2022 campaign with a yield of 305 q per hectare according to the Local Directorate of Agricultural Service.

The main objective of this study is to estimate the variation of soil organic carbon stock in the agricultural workshop of Mazgran at Mostaganem according to their occupations. To do this, we quantified the organic carbon according to the main land uses represented by citrus cultivation, namely: orange cultivation, grapefruit cultivation and lemon cultivation and spontaneous vegetation from the topsoil (0 - 30 cm) of the same study area.

## MATERIALS AND METHODS

**Study area.** The soils were collected on an experimental site (AW: Agricultural Workshop), located in the municipality of Mazagran, 4 km south of Mostaganem in the north-west of Algeria, its geographical coordinates are: 35°55′52″ N latitude and 0°05′21″ E longitude. The altitude varies between 125 and 151 m. The topography is relatively flat and limited to the north by the Mostaganem city, to the south by Hassi Mameche and Stidia, to the east by Ain Sidi Cherif and Mesra, and the west by the Mediterranean Sea reliefs. The AW occupies an area of 62.74 hectares, the area cultivated with citrus fruits since 1950 represents 2.80 hectares, or 4.6% of the total area occupied by citrus crop with 360 lemon trees, 135 orange trees and 10 grapefruit trees. The climate is semi-arid Mediterranean with a mean annual temperature is around 18, 3°C and mean annual rainfall of 425 mm (Figure 1) [26].

**Soil analysis.** Soil samples (0-30 cm) were collected from each point of the experimental site with three replicates according to the main land uses (citrus cultivations: (OC) orange cultivation, (GC) grapefruit cultivation, (LC) lemon cultivation and (SV) spontaneous vegetation) in Mars 2023. Finally, a total of 12 [3(OC) +3(GC) +3(LC) +3(SV)] soil samples were collected for the SOCS estimation.

It was interesting to determine other parameters (Table 1) to better understand the dynamics of soil organic carbon stock that could directly or indirectly influence it variation, such as: bulk density, total and active limestone, electrical conductivity, hydrogen potential, organic carbon content, and coarse element load.

The soil samples were dried at room temperature and passed through a 2 mm diameter sieve.

According to [36], the SOC stock was calculated as:

SOC stocks (Mg C $ha^{-1}$) = 0.1* C * BD * t*(1-CP)

Where SOC stock is a soil organic carbon stock (Mg C $ha^{-1}$), C is the carbon content (g C $kg^{-1}$ soil); BD is the bulk density (g $cm^{-3}$), t is the thickness of the soil horizon (cm) and CP is the coarse element load (g $g^{-1}$ of soil).

The C stocks were calculated for each soil sample for 0-30 cm depth where the organic matter concentrations and the microbial densities are the highest [5].

**Data analysis.** Correlation analysis was used to demonstrate the relationship between the SOCS and

the different physic-chemical parameters, this analysis was conducted by R Software [37].

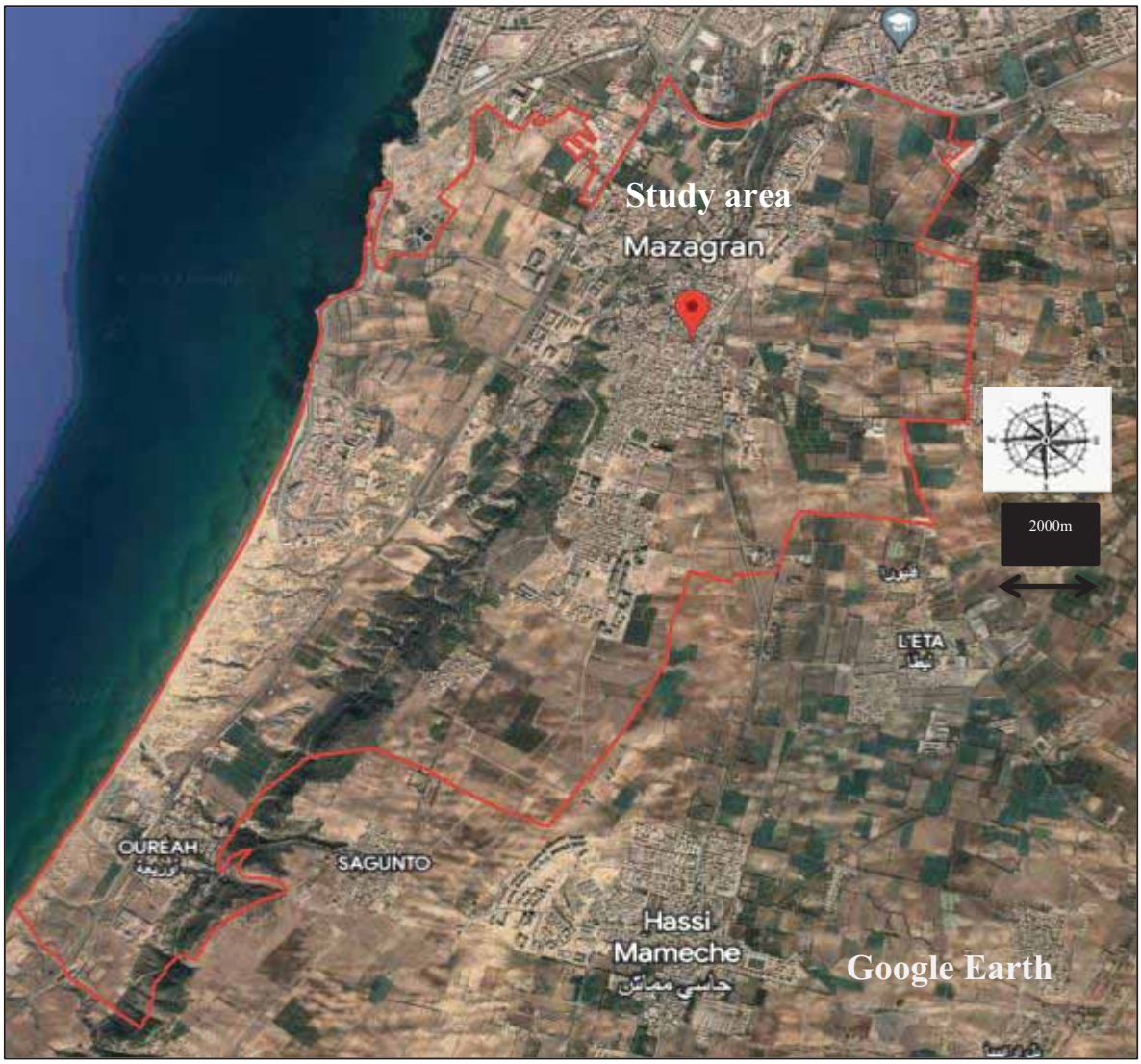



**FIGURE 1**
**Geographical location of the study area (AW Mazagran, Mostaganem).**

**TABLE 1**
**Different parameters influencing the SCOS.**

| Parameter | Method | Reference |
|---|---|---|
| Mineral fractions in % | Robinson pipette method, considered fractions being sand (50-2000 µm), silt (2-50 µm), and clay (0-2 µm). | [27-28]. |
| Total carbonate (Total $CaCO_3$ content) in % | Bernard calcimeter method using HCl acid. | [28]. |
| Active carbonate (Active $CaCO_3$ content) in % | Drouineau-Galet method by using ammonium oxalate. | [29]. |
| pH | Electrometric method 2:5 (m/v) by using pH meter (Starter 200, Ohaus). | [30-31]. |
| Salinity in µS $cm^{-1}$ | Electrical conductivity (EC) method 1:5 (m/v) by using a conductivity meter (Consort). | [32]. |
| Soil organic carbon (SOC) in % | The modified Walkley and Black method, based on the oxidation of OC by potassium dichromate $K_2Cr_2O_7$ in sulfuric acid. | [33]. |
| Bulk density (BD) in g $cm^{-3}$ and coarse element load (CP) in g $g^{-1}$ | The bulk density (BD) was determined by the direct sampling method, using a steel cylinder with a volume of 252.2 $cm^3$. The replicates of each soil sample were oven-dried for 24 h at 105 °C and sieved at 2 mm to remove coarse element load. | [34-35]. |

**TABLE 2**
**Mineral fractions of studied soils.**

| Soil samples Particles (%) | SV | OC | GC | LC |
|---|---|---|---|---|
| **Sands (S)** | 74 | 71 | 69 | 66 |
| **Fine silts (FS)** | 10 | 7 | 5 | 12 |
| **Coarse silts (CS)** | 7 | 10 | 12 | 15 |
| **Clays (C)** | 9 | 12 | 14 | 7 |
| **Texture Class** | **Loamy-sandy** | **Loamy-sandy** | **Loamy-sandy** | **Loamy-sandy** |

## RESULTS AND DICUSSION

**Variation of the studied parameters according to the soil occupations. Mineral fractions.** The analytical results (Table 2) showed that the four soils studied have very high proportions of sands (66% - 74%), but the difference is observed for the silt and the clay fractions, since the soil under GC has the highest rate of clays (14%) compared to the other three soil samples, while the soils under SV, OC and GC have almost the same percentages of silts (17%) and lower than that of the soil under LC (27%). According to triangle of textures [38], the four soils studied had the same textural class: loamy-sandy but with precise proportions for each fraction. In addition, they all had equal proportions of fine silts + clays (about 19%), therefore, the soil in the study area had a balanced texture.

**Soil bulk density.** Soil bulk density globally reflects the state of soil compaction and indirectly, the total porosity [39]. It is one of the most important parameters of soil structure [40]. The results showed that all the studied soils (Figure 2a) have an ideal bulk density for plant growth since they are less than 1.60 g $cm^{-3}$, around of 1.1 ± 0.065 g $cm^{-3}$, 1.22 ± 0.078 g $cm^{-3}$, 1 ± 0.058 g $cm^{-3}$ and 1.13 ± 0.061 g $cm^{-3}$ respectively for soils under SV, OC, GC and LC (Figure 2a). In addition, they had a favorable structure to root development since they had bulk densities less than 1.80 g $cm^{-3}$. According to [41], a high value of bulk density means that the voids are reduced and that the particles are strongly compacted, this means that our studied soils are light.

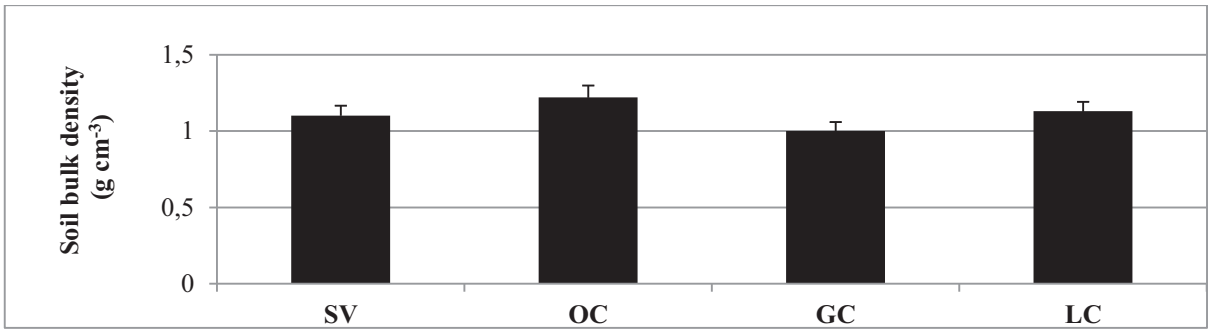
**Total and active limestone.** The result obtained indicated (Figure 2b) that all studied soils were slightly rich in total $CaCO_3$ (7.81 ± 0.140%, 7.63 ± 0.08%, 7.3 ± 0.025% and 9.23 ± 0.185%) and active $CaCO_3$ (1.5 ± 0.77%, 1.5 ± 0.62%, 1 ± 0.22% and 1 ± 0.07%) respectively for soils under SV, OC, GC and LC, so these were weakly calcareous soils in their entirety [42]. The fact of having found limestone in our soils was probably due to the presence of a limestone slab at depth, because according to [28], soils formed on limestone rocks are rich, even very rich in calcium. According to [43], it is generally considered that serious problems can begin to appear from active limestone contents close to 50% such as ferric chlorosis, this is the case for horticulture, arboriculture or viticulture, therefore, we can deduce that our soils are in the norm, and there is no risk of having problems linked to active limestone.

**Electrical conductivity.** According to the Figure 2 (c), the electrical conductivity results of the four soils studied varied between 119 ± 1.619 µS $cm^{-1}$ and 205 ± 2.971 µS $cm^{-1}$. The saline concentration of the nutrient solution plays a major role in the water supply of the plant. It determines the osmotic pressure of the solution. This must be lower than the osmotic pressure of the cell juice, so that the water can diffuse from the solution to the plant. The most immediate consequence of excessive salt concentration is root injury followed by plant wilting [44].

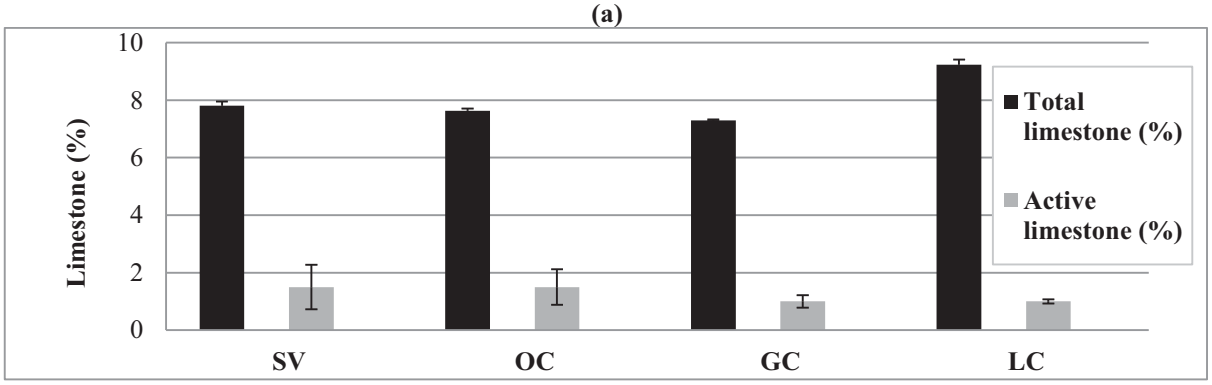
According to [45], a soil with an EC ≤ 500 µS $cm^{-1}$, is an unsaline soil, and the effect of the latter on the yield is negligible, which is completely consistent with the results we obtained.

**pH.** The results obtained (Figure 2d) indicated that all the soil samples were basic [46], with slight variations: between 7.81 ± 0.064 and 8.09 ± 0.006 for the soils under GC and LC respectively, and maximum (between 8.26 ± 0.017 and 8.24 ± 0.029) for the soils under SV and OC respectively. The pH, close to 8, is imposed by the presence of calcium and magnesium carbonates and cannot be modified by agricultural practices. In the absence of carbonates, the soil pH is neutral or acidic [47], but apparently our soils were basic and slightly calcareous, so this may be due to the quality of irrigation water which may be high in sodium. pH is one of the strong predictors of bacterial community composition and diversity [48].

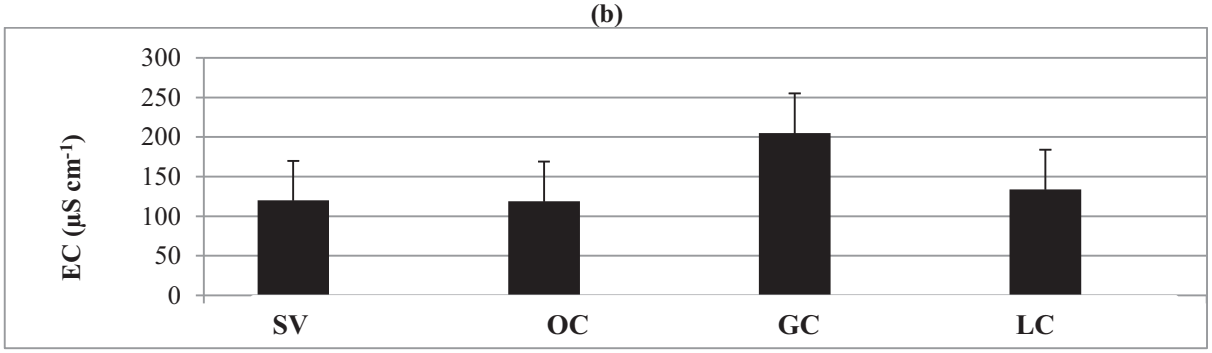
**Soil organic carbon.** Soil organic matter, especially SOC promotes a wide variety of habitats for microorganisms in soil [11]; which, through its activities, produce humus without which a soil becomes dead, unsuitable for plants growth [49], or degrades and then transforms it into a mineral form that can be absorbed by plants [50]. However, SOC is a cost-effective and informative indicator of soil quality and agriculture sustainability as it affects soil physical, biological and chemical properties



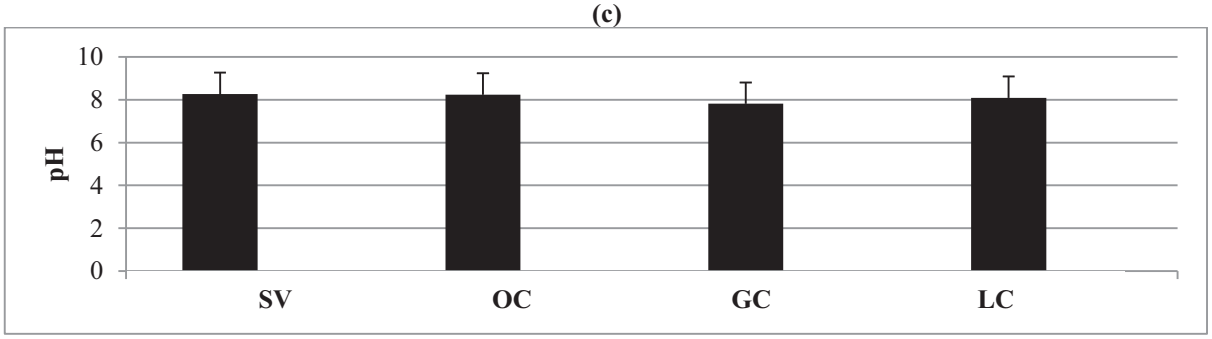

(a)



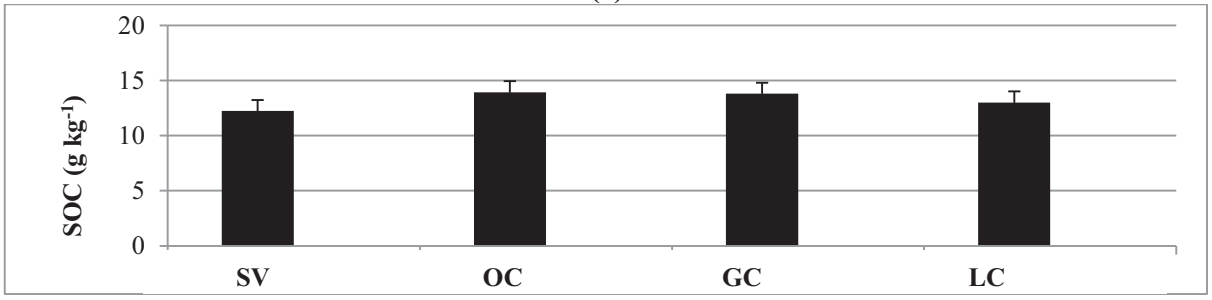

(b)




(c)




(d)




(e)

**FIGURE 2**
**Variation of (a) bulk density (g cm$^{-1}$), (b) limestone (%), (c) EC (µS cm$^{-1}$), (d) pH, (e) SOC (g kg$^{-1}$) according to the soil occupations.**

According to Figure 2 (e), the results obtained showed that our four soils have organic matter rates greater than 2% and less than 4%, so the soils studied are average in organic matter [42]. Moreover, it is found that the soils under OC, GC and LC had a higher stock of CO than that of the soil under SV, with values between 13 ± 0.7 g $kg^{-1}$, 13.8 ± 1.21 g $kg^{-1}$ and 13.93 ± 0.81 g $kg^{-1}$ respectively, and the minimum value observed in the soil under SV (12.23 ± 1.21 g $kg^{-1}$). The presence of the crop in the soil has a stimulatory effect for the microbial biomass because the exudation or the root production provides easily usable compounds which are at the origin of the stimulation of the microbial density, which according to [51] are constituted by an easily biodegradable labile material. However, it has been found that the presence of living roots in soils under citrus crop, leading to root exudation and rhizodeposition, is the source of microbial stimulation and its activity, which is manifested by an increase in biodegradation and mineralization [52-53].

**Variation of SOCS**. No previous studies on soil organic carbon sequestration under citrus crop were found. According to [22], SCOS is influenced by agricultural land use, cultivation practices, soil depths and soil physico-chemical parameters.

**Variation of the SCOS according to the soil occupations.** According to [54], the type of vegetation influences the organic matter content of agricultural soils. Figure 3 showed the effect of the presence of the crop on the SCOS, the average stock is 45.3 ± 11.45 Mg C $ha^{-1}$ for all 12 soil samples in the study area, characterized by the loamy-sandy texture, taken at 30 cm depth with 40.52 ± 4.48 Mg C $ha^{-1}$, 51.26 ± 6.26 Mg C $ha^{-1}$, 46.66 ± 4.69 Mg C $ha^{-1}$ and 42.76 ± 2.23 Mg C $ha^{-1}$ for soils under SV, OC, GC and LC respectively.

The SOCS varied significantly ($p<0.05$) between the different soils under citrus cultivation and the soil under spontaneous vegetation , with the highest value recorded for the soil under orange cultivation (51.26 ± 6.26 Mg C $ha^{-1}$), followed by the soil under grapefruit cultivation and the soil under lemon cultivation, the lowest stock recorded for the soil under spontaneous vegetation (40.52 ± 4.48 Mg C $ha^{-1}$), therefore the roots of the plants essential contributor to the formation of soil organic matter [55], according to [56], this variation could also come from the difference in plant inputs, which differ in quantity and quality between species [57-58], as well as from the composition of aboveground and belowground plant tissues between species [59].

**Variation of SCOS according to physico-chemical parameters.** The result of the correlation analysis indicated that SOC stock has: positive correlations with (i) bulk density, with (ii) soil organic carbon, with (iii) clays and with (iv) calcium carbonates (active).

Organic matter improves soil structure because the resulting humus prevents compaction into a solid mass and promotes the granulation of particles, making soils lighter, more porous and better aerated [60-61] and promotes the storage of organic carbon in the soil [62].

The positive correlation between clay and SOCS could be explained by the characteristics of clay, which acts as an aggregation factor, binding particles together and influencing OC decomposition and turnover [28].

[63], who discussed the positive correlation between calcium carbonates (total and active) and SCOS. Contrary to the results of several authors, notably [64-66] who found a negative correlation for soils under grassland.

**Variation of SOCS with depth.** The highest stock (0-30 cm) recorded for the soil under OC (51.26 ± 6.26 Mg C $ha^{-1}$), this value was not far from that found for Spanish soils under the same climatic conditions with 50.8 ± 33.7 Mg C $ha^{-1}$ [67] and for French arable land with 51.6 Mg C $ha^{-1}$ [25,68]. Moreover, this value was higher than the value recorded by: (i) [69] for all Tunisian soils with an average of 26.12 Mg C $ha^{-1}$, (ii) to those found by [70] for soils under arboriculture in Morocco with an average of 38 Mg C. $ha^{-1}$ and (iii) to those recorded by [23] Benslimane et al. (2023) for the soils of the plain of Sidi Bel Abbes (44.10 Mg C $ha^{-1}$ for olive cultivation, 32.46 Mg C $ha^{-1}$ for cereal cultivation and 36.18 Mg C $ha^{-1}$ for viticulture), and still above the global average value for shrubby areas 46.57 Mg C $ha^{-1}$ but below the global average value for wooded areas with 66.84 Mg C $ha^{-1}$ [71].

## CONCLUSION

The present study focused on the estimation of carbon sequestration in agricultural soils according to their occupations under semi-arid Mediterranean conditions at the Mazagran Agricultural Workshop in Mostaganem in the topsoil (0 - 30 cm). The results showed that SOCS would vary significantly ($p<0.05$) between the different modes of land use, with an average of 45.3 ± 11.45 Mg C $ha^{-1}$ for all 12 samples taken, with 51.26 ± 6.26 Mg C ha-$^{1}$ under orange cultivation, 46.66 ± 4.69 Mg C $ha^{-1}$ under grapefruit cultivation and 42.76 ± 2.23 Mg C $ha^{-1}$ under lemon cultivation, compared with 40.52 ± 4.48 Mg C $ha^{-1}$ under spontaneous vegetation.

Vegetation mainly conditions the input of organic matter to the soil over time and space, particularly arboriculture, in order to improve soil fertility, soil erosion resistance and preserve soil biodiversity through the root system, root exudation and rhizodeposition.

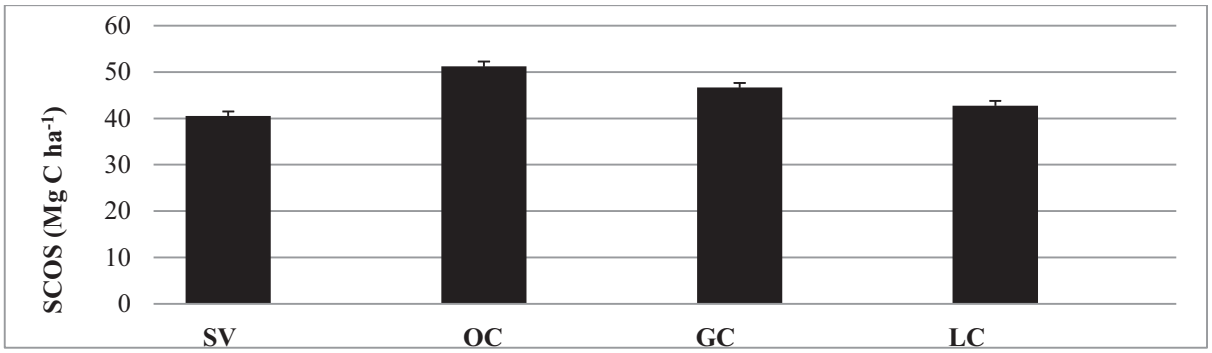



**FIGURE 3**
**Variation of SOCS according to soil occupations.**

The data generated by this study will be useful and contribute to the design of land management strategies to better conserve existing organic capital and increase carbon sequestration in agricultural soils in arid and semi-arid areas.

It is recommended that a comparative study be carried out with several types of arboriculture, such as: citrus cultivation, viticulture, olive cultivation, etc., in order to highlight the mechanisms of organic matter formation in relation to the length of living roots and according to different depths (0-15cm, 15-30cm, 30-45cm, 45-100cm).

## ACKNOWLEDGEMENT

Particular thanks are addressed to Dr. Faraoun Fatiha for the theme proposal in the Environmental Sciences Department of Sidi Bel Abbes University.

All my gratitude and thanks are addressed to Mr. Kaddour Azzedine, Director of the Agricultural Workshop (Mazagran) for having allowed us to carry out my study in the best conditions.

My sincere thanks and my deep appreciation for all those who contributed to the realization of this manuscript, Pr. Rezzoug Wafaa, Mrs. Benharira Sabrina and Mrs. El Kebaili Asma

Special thanks are also addressed to the Agronomy Department of Mostaganem University for facilitating access to the soil analysis laboratory.

Author has declared that no competing interests exist. The research was funded by the personal efforts of the author.

## REFERENCES

[1] Le Quéré, C., Moriarty, R., Andrew, R.M., Peters, G.P., Ciais, P., Friedlingstein, P., Jones, S.D., Sitch, S., Tans, P., Arneth, A., Boden, T.A., Bopp, L., Bozec, Y., Canadell, G.P., Chini, L.P., Chevallier, T., Cosc, C.E., Harris, I., Hoppema, M., Houghton, R.A., House, J.I., Jain, A.K., Johannessen, T., Kato, E., Keeling, R.F., Kitidis, V., Goldewijk, K.K., Kove, C., Landa, C.S., Landschutzer, P., Lenton, A., Lima, I.D., Marland, G., Mathis, J.T., Metzl, N., Nojiri, Y., Olsen, A., Ono, T., Peng, S., Peters, W., Pfeil, B., Poulter, P., Raupach, M.R., Regnier, P., Rodenbeck, C., Saito, S., Salisbury, J.E., Schuster, U., Schwinger, J ., Seferian, R., Segschneider, J., Steinhoff, T., Stocker, J.E., Sutton, A.J., Takahashi, T., Tilbrook, B., van der Werf, G.R., Viovy, G.R., Wang, Y.P., Wanninkhof, R., Wiltshire, A. and Zeng, N. (2015) Global Carbon Budget 2014. Earth System Science Data. 7, 47-85.

[2] Jobbágy, E.G. and Jackson, R.B. (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423- 436.

[3] Six, J., Connant, R.T., Paul, E.A. and Paustian, K. (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil. 241, 155–176.

[4] Derrien, D., Dignac, M.F., Basile-Doelsch, I., Barot, S., Cécillon, L., Chenu, C., Chevallier, T., Freschet, G.T., Garnier, P., Guenet, B., Hedde, M., Klumpp, K., Lashermes, G., Maron, P.A., Nunan, N., Roumet, C. and Barré, P. (2016) Storing C in soils: What mechanisms, what agricultural practices, what indicators?. Soil Study and Management (in French). 23, 193–223.

[5] Bernoux, M. and Chevallier, T. (2013) Carbon in dry areas. Multiple essential functions. CSFD thematic files). Agropolis International. (Avaible:file:///C:/Users/User/Downloads/Fiche_10_CSFD_Francais%20(2)) (in French). (Accessed Date: 11/06/2018). 10, 1-44.

[6] Blanchart, E. and Trap, J. (2020) Intensifying soil ecological functions for sustainable provision of ecosystem services in agriculture. Soil Study and Management. (Available: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers20-04/010077640.pdf). (Accessed Date: 19/12/2021). 27, 121-134.

[7] Arrouays, D., Balesdent, J., Germon, J.C., Jayet, P.A, Soussana, J.F. and Stengel, P. (2002) Contribution to the control of the greenhouse effect. Carbon storage in French agricultural soils? Collective scientific expertise. INRA report. Paris: National Institute for Agronomic Research (Inra). (Available : https://hal.inrae.fr/hal-02832401/document). (Accessed Date: 23/11/2019). (in French). 332 P.

[8] Jacobson, M., Charlson, R.J., Rodhe, H. and Orians, G.H. (2000) Earth System Science: From Biogeochemical Cycles to Global Changes. Academic Press. 202P.

[9] Scharlemann, J.P., Tanner, E.V., Hiederer, R. and Kapos, V. (2014) Global soil carbon: understanding and managing the largest terrestrial carbon pool. Carbon Manag. 5, 81-91.

[10] Arrouays, D., Feller, C., Jolivet, C., Saby, N., Andreux, F., Bernoux, M. and Cerri, C. (2003) Estimation of soil organic carbon stocks at different spatial and temporal scales.Soil Study and Management. (Available :https://core.ac.uk/download/pdf/39844583.pdf). (in French). (Accessed Date: 23/11.2019). 10(4), 347-355.

[11] Tosser, V., Eglin, T., Bardy, M., Bessonet, A. and Martin, M. (2013) Assessment of organic carbon stocks in cultivated soils in France Application of the Tier 1 methodology of the Intergovernmental Panel on Climate Change (IPCC) to the sites of the Soil Quality Measurement Network (SQMN). Soil Study and Management (in French). 21, 7-23.

[12] Minasny, B., Malone, B., McBratney, A., Angers, D., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Chengg, K., Das, B., Fielda, D., Gimona, A., Hedley, C., Hong, S.Y., Mandal, B., Marchant, B., Martin, M., McConkey, B., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van-Wesemael, B., Winowiecki, L. (2017) Soil carbon 4 per mille. Geoderma. 292, 59-86.

[13] Brahim, N., Blavet, D., Gallali, T. and Bernoux, M. (2011) Application of structural equation modeling for assessing relationships between organic carbon and soil properties in semiarid Mediterranean region. International Journal of Environmental Science and Technology. 8(2), 305-320.

[14] Jones, A., Breuning-Madsen, H., Brossard, M., Dampha, A., Deckers, J., Dewitte, O., Gallali, T., Hallett, S., Jones, R., Kilasara, M., Le Roux, P., Micheli, E., Montanarella, L., Spaargaren, O., Thiombiano, L., Van Ranst, E., Yemefack, M. and Zougmore, R. (2013) Soil Atlas of Africa. European Commission, Publications Office of the European Union, Luxembourg. 176P.

[15] Lefèvre, C., Rekik, F., Alcantara, V. and Wiese, L. (2017) Soil organic carbon: An invisible wealth. Food and Agriculture Organization of the United Nations, Rome, Italy. (in French). 98P.

[16] Arabi, M. and Roose, E. (2004) Influences of production system and soil on water erosion, runoff, carbon stock and losses by erosion in Mediterranean mountain areas (Medea, Algeria). Erosion Network Bulletin. 22, 166-175.

[17] Boutkhil, M., Amer, H. and Eric, R. (2004) Effect of management systems on water erosion and carbon stock in the beni chougrane-algeria Mountains. Erosion Network Bulletin. (Available : https://www.researchgate.net/publication/40438350). (Accessed Date:). (in French). 23, 416-430.

[18] Morsli, B., Mazour, M., Mededjel, N., Halitim, A. and Roose, E. (2004) Effect of management systems on erosion and carbon stock in the western Tell Mountains of Algeria. (Available : http://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers11-11/010053285.pdf). (Accessed Date: 04/05/2021). (in French).

[19] Daroui, A., Weisskopf, P., Oberholzer, H.R. and Benslama, M. (2018) Evaluation of total Carbon and total Nitrogen content in two Algeria regions under the effect of three tillage systems. Int. J. Biosci. 12(1), 410-416.

[20] Bounouara, Z., Chevallier, T., Balesdent, J., Toucet, J., Sbih, M., Bernoux, M., and Bensaid, R. (2017) Variation in soil carbon stocks with depth along a toposequence in a sub-humid climate in North Africa (Skikda, Algeria). Journal of Arid Environments. 141, 25-33.

[21] Merabtene, M.D., Faraoun, F., Mlih, R., Djellouli, R., Latreche, A. and Bol, R. (2021) Forest Soil Organic Carbon Stocks of Tessala Mount in North-West Algeria- Preliminary Estimates. Front. Environ. Sci. 8, 520284.

[22] Benouadah, S., Faraoun, F. and Oulbachir, K. (2022) Incidence of manure amendment on soil organic stock under semi-arid environment. Fresen. Environ. Bull. 31(11), 10892-10902.

[23] Benslimane, Z., Faraoun, F. and Latreche, A. (2023) Land use and impact on soil organic carbon stock in semi-arid region of Algeria. Al-Qadsiyah Journal for Agriculture Sciences (QJAS). 1(13), 36-49.

[24] Chevallier, T., Fujisaki, K., Roupsard, O., Guidat, F., Kinoshita, R., Filho, V., Lehner, P. and Albrecht, A. (2019) Short-range-order minerals as powerful factors explaining deep soil organic carbon stock distribution: the case of a coffee agroforestry plantation on Andosols in Costa Rica. Soil. 5, 315–332. (Avaible at: http://agritrop.cirad.fr/594924/1/Chevallier%20Soil%202019%20Soil%20andic%20properties%20Costa%20Rica%20) (Accessed date: 25/05/2022).

[25] Arrouays, D., Deslais, W. and Badeau, V. (2001) The carbon content of topsoil and its geographical distribution in France. Soil use and Management. 17(1), 7-11.

[26] ANIREF (National Agency for Intermediation and Land Regulation). (2018) Monograph of the Wilaya of Mostaganem (in French). 31P.

[27] Gee, G. and Or, D. (2002) Particle size analysis. In 'Methods of soil analysis, Part 4. Physical methods'. Soil Science Society of America. Book Series. 5, 255-293.

[28] Schvartz, C., Muller, J-C. and Decroux, J. (2005) Agriculture and fertilization. in Guide to rational fertilization. Agricultural France, Comifer. (in French). ISBN: 2-85557-120-0, 473P.

[29] Drouineau, G. (1942) Rapid determination of soil active limestone: new data on separation and the 477 natures of limestone fractions. Ann. Agron. (in French). 12, 441-450.

[30] Dicova, C. (1997) Crop fertilization and treatment equipment. Tec & Doc, Ed. Lavoisier. Paris (in French).
276P.

[31] Mathieu, C., Pieltain, F. and Jeanroy, E. (2003) Soil chemical analysis: Selected methods.Tec & Doc, Ed. Paris. (in French). 233P.

[32] Calvet, R. (2003) The soil, Properties and functions: Constitution and structure, phenomena at the interfaces. Vol1. Agricultural France, Ed. Lavoisier. Paris. (in French). 457P.

[33] Gillman, G.P., Sinclair, D.F. and Beech, T.A. (1986) Recovery of organic carbon by the walkley and black procedure in highly weathered soils. Commun. Soil Sci. Plant. Anal. 17 (8), 885–892.

[34] Grosbellet, C. (2008) Evolution and effects on soil structure of organic matter brought in large quantities. INRA, Sagah Angers University, Paris. (in French).81-88.

[35] Blake, G.R. and Hartge, K. (1986) Bulk density. Methods Soil Analysis. 5, 363–375.

[36] FAO (2018) Measuring and modelling soil carbon stocks and stock changes in livestock production systems – Guidelines for assessment (Draft for public review). Livestock Environmental Assessment and Performance (LEAP) Partnership. FAO, Rome, Italy, 64.

[37] Core Team, R.R. (2020) A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 55.

[38] Gobat, J.M., Aragno, M. and Matthey, W. (2010) The living soil. 3rd Ed. Polytechnic and University Press Lausanne. Italy. (in French). 817P.

[39] Alongo, S. and Kombele, F. (2009) Evolution of the apparent density and the C / N ratio of the soil under the exostic and local varieties of cassava under the natural conditions of Kisangani (DR Congo). Annals of the Faculty Institute of Agronomic Sciences of Yangambi. (in French). 1, 197-214.

[40] Chauvel, A. (1977) Research on the transformation of ferrallitic soils in the tropical zone with contrasting seasons. Office for Scientific and Technical Research Overseas, French. (in French). 497-511.

[41] Mermound, A. (2010) Soil physics course. Lausanne Federal Institute of Technology, Ouagadougou and Lausane (in French). 125-154.

[42] ITA Agricultural Technology Institute of Mostaganem-Algeria (1977) Soil laboratory: Physical and chemical soil analysis methods 3rd and 4th year. (in French). 78P.

[43] Pousset, J. (2002) Green manures and soil fertility. Agridecisions, 2nd Ed. Paris. (in French). 305P.

[44] Blanc, D. (1987) Above-ground crops. INRA, 2nd Ed. Paris.(in French). 413P.

[45] Durand, J.H. (1983) Irrigable soils, soil study. University Press of France. Agency for Cultural and Technical Cooperation, Ed. Paris. (in French). 339P.

[46] Baize, D., Duval, O. and Richard, G. (2013) Soils and their structures: Observations at different scales. QUAE, Ed. Versailles. (in French). 285P.

[47] Stengel, P., Gelin, S. (1998) Soil : fragile interface. INRA, Ed. Paris. (in French). 213P.

[48] Faugier, A. (2010) Bacterial diversity of soils: access to populations with minority numbers "the rare biosphere". Electronic, Electro-Technical and Automatic Doctoral School. Lyon. 173P.

[49] Doucet, R. (2006). Climate and agricultural soils Berget A.C (ed). Canada. (in French). 172-173.

[50] Dridi, B. and Toumi, C. (1999) Influence of organic amendments and sludge inputs on the properties of a cultivated soil. Soil Study and Management. (in French). 6(1), 7-14.

[51] Lynch, J. (1982) Interaction between bacteria and plants in the root environment. Bactéria and Plants, Ed. Academic Press. London. 123P.

[52] Oulbachir, K., Zoubeidi, M., Kouadria, M and Bouchenafa, N. (2014) Conservation practices agro systems for sustainable development. Mater Environment Science Journal. (in French). 5, 2.
[53] Benouadah, S., Oulbachir, K., Benaichata, L., Miara, M.D. and Snorek, J. (2020) Evolution of the microbial population of a cultivated soil with organic matter input under semi-arid con-ditions (Tiaret, Algeria). Ukrainian Journal of Ecology. 10(3), 28-35.
[54] Jaouadi, J., Aichi, H., Ben Hassine, H., Hatira, A. and Balesdent, J. (2019) Carbon Turnover in a Sorghum-cultivated Semi-arid Mediterranean Soil Profile. Communications in Soil Science and Plant Analysis. 50(19), 2457-2468.
[55] Poirier, V., Roumet, C. and Munson, A. D. (2018) The root of the matter: linking root traits and soil organic matter stabilization processes. Soil Biology and Biochemistry. 120, 246-259.
[56] Guo, L.B., Halliday, M.J., Siakimotu, S.J.M. and Gifford, R.M. (2005) Fine root production and litter input: its effects on soil carbon. Plant and Soil. 272(1), 1-10.
[57] De Deyn, G.B., Cornelissen, J.H. and Bardgett, R.D. (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters. 11(5), 516-531.
[58] Soltanpour, P.N. and Schwab, A.P. (1977) A new soil test for simultaneous extraction of micro and macro nutrients in alkaline soil. Commun. Soil. Sci. Plant. Anal. 9, 195-207.
[59] Kögel-Knabner, I. (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry. 34(2), 139-162.
[60] Doucet, R. (2006) Climate and agricultural soils Berget A.C (ed). Canada. (in French). 172-173.
[61] Deksissa, T., Short, I. and Allen, J. (2008) Effect of soil amendment with compost on growth and water use efficiency of Amaranth. In: Proceedings of the UCOWR/NIWR Annual Conference, International Water Research Challenges for the 21st Century and Water Resources Education, July 22-24, 2008, Durham, NC, USA. 152P.
[62] Moghiseh, E., Heidari, A. and Ghannadi, M. (2013) Impacts of deforestation and reforestation on soil organic carbon storage and $CO_2$ emission. Soil Environ. (Avaible at : http://www.se.org.pk/) (Accessed date: 10/10/2019). 32(1), 01-13.
[63] Rowley, M.C., Grand, S. and Verrecchia, É-P. (2018) Calcium-mediated stabilisation of soil organic carbon. Biogeochemistry. 137(1), 27-49.
[64] Yang, S., Cammeraat, E., Jansen, B., Cerli, C. and Kalbitz, K. (2016) Organic carbon stabilization of soils formed on acidic and Calcaric bedrocks in Neotropical alpine grassland, Peru. EGU General Assembly 2016, held 17-22 April, 2016 in Vienna, Austria. 312P.
[65] O'Brien. S.L., Jastrow, J.D., Grimley, D.A. and Gonzalez-Meler, M-A. (2015) Edaphic controls on soil organic carbon stocks in restored grasslands. Geoderma. 251–252, 117–123.
[66] Li, D., Wen, L., Yang, L., Luo, P., Xiao, K., Chen, H., Zhang, W., He, X., Chen, H. and Wang, K. (2017) Dynamics of soil organic carbon and nitrogen following agricultural abandonment in a karst region. J. Geophys. Res. Biogeosci. 122, 230–242.
[67] Rodríguez-Murillo, J.C. (2001) Organic carbon content under different types of land use and soil in peninsular Spain. Biology and Fertility of Soils. 33(1), 53-61.
[68] Pellerin, S., Bamière, L., Launay, C., Martin, R. and Réchauchere, O. (2019) Storing carbon in French soils, what potential with regard to the 4 per 1000 objective and at what cost? Summary of the study report, INRA (France). (in French). 114 P.
[69] Brahim, N., Bernoux, M., Blavet, D. and Gallali, T. (2010) Tunisian soil organic carbon stocks. International Journal of Soil Science.5(1), 34-40.
[70] Sabir, M., Sagno, R., Tchintchin, Q., Zaher, H. and Benjelloun, H. (2020) Evaluation of organic carbon stocks in soils in Morocco. in Soil Carbon in Africa. Ed IRD. Maroc. (in French). 90P.
[71] FAO and CMCC (2017) Global Soil Organic Carbon Database (at 30 arcsec). Rome: Food and Agriculture Organization of the United Nations and Euro- Mediterranean Center on Climate Change Foundation.

**Received:** **02.08.2023**
**Accepted:** **15.08.2023**

**CORRESPONDING AUTHOR**

**Benouadah Salima**
Laboratory of Biodiversity, Water and Soil Conservation, Faculty of Natural and the Life Sciences,
Abdelhamid Ibn Badis University,
BP 188/227 Mostaganem, 27000, Algeria

e-mail: salima.benouadah@univ-mosta.dz

-© by PSP Volume 32– No. 09/2023 pages 3007-3018 Fresenius Environmental Bulletin

# ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS USED FOR DIABETES TREATMENT IN THE REGION OF SIDI BEL ABBES IN ALGERIA

**Nadjia Fertout-Mouri[1,*], Khalida Zemri[2], Assia Ghezali[2], Meryem Belabbas[3], Ouissem Berrached[4]**

[1]Laboratory of Plant Biodiversity: Conservation and Valorization, Faculty of Nature and Life Sciences, Djillali Liabès University of Sidi Bel Abbes, Algeria
[2]Department of Biology, Faculty of Nature and Life Sciences, University of Djillali Liabès, Sidi Bel Abbes, Algeria
[3]Laboratory of Beneficial Microorganisms, Functional Food and Health (LMBAFS), Faculty of Nature and Life Sciences, Djillali Liabès University of Sidi Bel Abbes, Algeria
[4]Faculty of Nature and Life Sciences, University of Djillali Liabès, Sidi Bel Abbes, Algeria

## ABSTRACT

Diabetes stands as a profoundly critical public health concern. In light of this, the primary objectives of the present study were twofold: firstly, to identify the plants employed in the treatment of diabetes, and secondly, to characterize the therapeutic applications of these plants based on the ethnobotanical data collected at the population level within the Sidi Bel Abbes region.

This ethnobotanical survey was conducted using questionnaire sheets, ensuring the inclusion of a representative sample from the population, consisting of 511 respondents of both genders. The findings of the ethnobotanical study indicated that female exhibit a substantially higher usage of medicinal plants compared to male, particularly among brides. Additionally, it was observed that older female, specifically those aged over 60 years, who often have limited literacy, were the most frequent users of traditional medicine.

A total of fifty plants were identified for their use in diabetes treatment. Among these, leaves, seeds, and stems emerge as the most commonly employed plants parts. Regarding the methods of preparation, decoction and infusion were the predominant modes of use. The floristic investigation revealed that the recorded species belong to 29 distinct botanical families. The Lauraceae, Asteraceae, Fabaceae and Rosaceae families were the most frequently utilized for medicinal purposes in the treatment of diabetes.

**KEYWORDS:**
Diabetes, Ethnobotanical survey, Traditional medicine, Medicinal plants, Questionnaires, Sidi Bel Abbes

## INTRODUCTION

Throughout history, humans have relied on the resources present in their environment to alleviate, treat, and cure numerous diseases. The practice of employing natural resources for medicinal purposes has been an integral part human existence for a considerable period [1].

In traditional medicine, medicinal plants are esteemed for their unique properties that offer significant benefits to human health. The prevalent methods of using these plants include decoction, maceration, and infusion. Various parts of plants, such as roots, leaves, and flowers, are harnessed either individually or in combination to achieve therapeutic effects. [2]

Nowadays, the knowledge possessed by traditional healers is gradually diminishing, leading to its potential extinction. To address this concern, ethnobotany and ethnopharmacology are actively engaged in the global pursuit of identifying plants believed to possess medicinal properties. As a result, it becomes the responsibility of modern scientific research to further investigate and validate the therapeutic uses of these plants [3].

Diabetes has become a major global public health issue in recent years. In Algeria, the prevalence of diabetes is increasing, impacting both urban and rural populations [4]. Additionally, diabetes-associated macro-angiopathic complications are exacerbated when hypertension coexists with the condition [5].

Given these facts, we performed an ethnobotanical survey in the Sidi Bel Abbes region with the aim to identify medicinal plants used to treat diabetes and hypertension. This survey aims to meticulously document all pertinent information concerning the diverse therapeutic practices employed by the local population in addressing these two health conditions. The gathered data will contribute to advancing our understanding of traditional medicinal practices and potentially uncover valuable resources for the treatment and management of diabetes and hypertension in the region.

## MATERIALS AND METHODS

A systematic inventory was conducted to identify the different plant species that constitute a medicinal floristic heritage in order to recognize the potential of medicinal plants used to treat diabetes in the region of Sidi Bel Abbes.

**Study area.** The wilaya (province) of Sidi Bel Abbes is situated in the southwestern region of the Algerian tell. Positioned in the north –western part of the country, it holds a strategically central location and encompasses approximately 15% of the regional territory, making it a substantial area that spans a cadastral surface of 915,063 km2 [6].

**Ethnobotanical study.** The data for the present study were collected through interviews with individuals who were either born into or have lived within conservative families that have a long-standing tradition of using medicinal plants to treat various diseases.

**Survey sites.** the ethnobotanical survey was conducted in the year 2021 across eight municipalities (as illustrated in figure 1 and summarized in table 1). The survey targeted communities with individuals who possess not only knowledge of medicinal plants but also insights into their practical use. The data collection process involved direct individual interviews, which was dialectal Arabic, or in French, depending on the educational background of each respondent. The respondents provided comprehensive responses to the questions, contributing to a detailed understanding of the traditional medicinal practices.

To ensure a high degree of objectivity of the data collected, the survey was performed using questionnaire sheets adapted to the objective of the current study. They are designed to collect as much information as possible about the age, gender, intellectual level and family situation of each respondent. Additionally, data concerning applications, parts used and preparation techniques were also collected.

**Population surveyed.** The surveyed population consisted of 511 individuals, with 364 identified as female and 147 as male. These individuals have been categorized into the following groups:

A random sample of 446 Individuals who utilize medicinal plants has been selected, comprising individuals from various occupation such as farmers, civil servants, traders, etc;

Additionally, 60 herbalists and 5 healers have been purposefully selected according to their esteemed reputation and extensive experience in the domain of utilizing plants in traditional medicine.

**Identification of species.** Upon the completion of these surveys, a comprehensive summary table was compiled, encompassing all the data collected. The identification of species, including both botanical names and vernacular names, was achieved through the use of several reference sources [7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23].

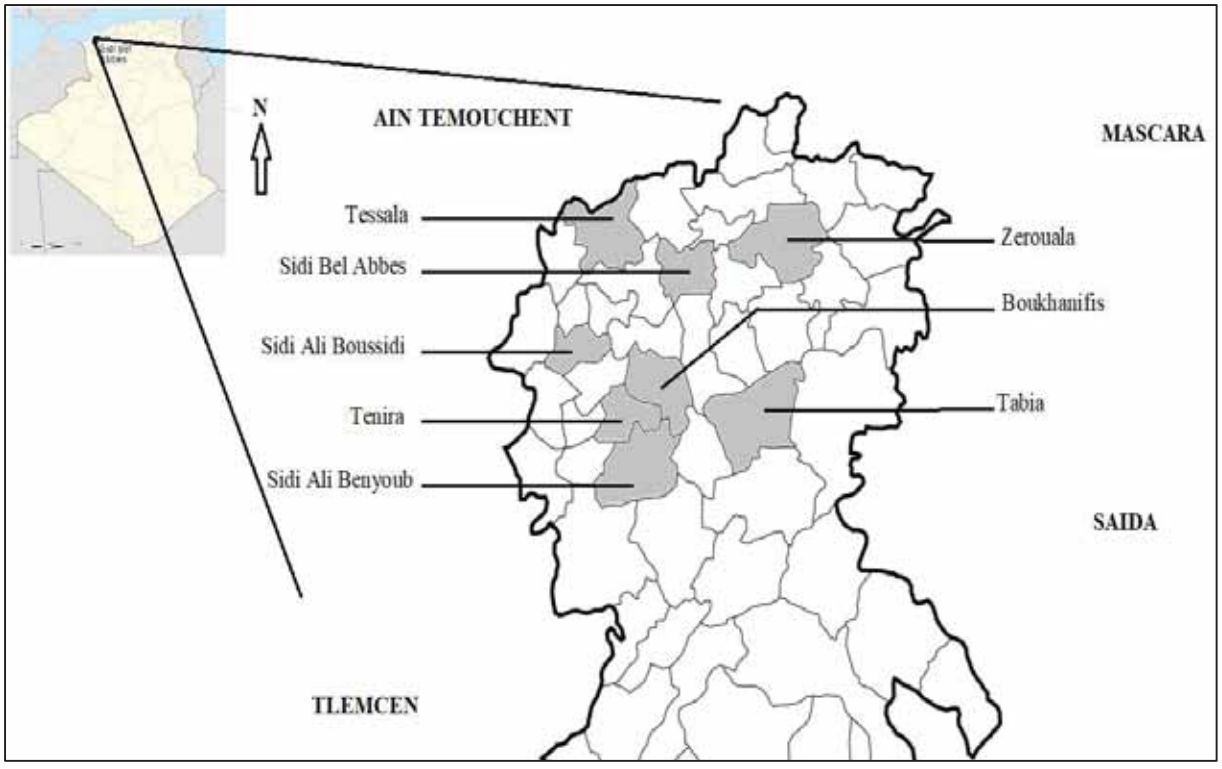



**FIGURE 1**
**Survey sites in Sidi Bel Abbes region**

**TABLE 1**
**Distribution of surveys among different municipalities.**

| municipalities | Survey number |
|---|---|
| Zaroula | 31 |
| Sidi Bel Abbes city | 197 |
| Tanira | 39 |
| Sidi Ali Benyoub | 51 |
| Tassala | 53 |
| Sidi Ali Boussidi | 61 |
| Boukanefis | 38 |
| Tabia | 41 |
| **Total surveys** | **511** |

**Data processing.** Following the conclusion of the ethnobotanical survey and the analysis of the questionnaires, the collected data was entered, processed, and subjected to analysis using Microsoft Excel. A descriptive analysis was performed to categorize the various users of medicinal plants identified based on the information gathered on the profiles of informants. This analysis involves examining factors such as: age, sex, and intellectual level and family situation of the individuals participating in the survey. Subsequently, the results obtained from both quantitative and qualitative data were described in terms of numbers and percentage. Excel was used to create graphical representations of the data. Furthermore, the phytotherapeutic citation index (PCI) was calculated to emphasize the plants that are used frequently than the others**.** The PCI was determined using the following formula: PCI = (n/N)*100 (where n: number of citations of the species and N: total number of citations of all species recorded in the survey).

## RESULTS AND DISCUSSION

**Ethnobotanical analysis. Gender of users.** The results of the survey indicate that among the 511 respondents, female were the primary users of medicinal plants, accounting for 71.23% of the total, while male constituted 28.77% (as depicted in figure 02). These findings suggest that female play a significant role in preserving traditional phytotherapeutic knowledge and are more inclined to benefits from the medicinal properties of plants within the family, female often served as the first line of assistance in providing medicinal treatments to their children. Their position within the family structure enables them to maintain continuous interactions experienced individuals, particularly seniors, who possess valuable knowledge in this field. Our results align with and corroborate findings from previous studies conducted in Algeria [24] and Morocco [25, 26]

**Family situation.** the survey revealed that the employment of medicinal plants was notably higher among married individuals, constituting 90.41% of all responders, while single individuals accounted for only 9.59% (as illustrated in figure 03). This disparity can be attributed to the fact that married female often turn to medicinal plants to alleviate various ailments and prevent the onset of infections of diverse origins. Through these practices, they can mitigate the expenses associated with doctor consultations and the purchase of pharmaceutical drugs. Moreover, younger individuals showed more reluctance towards the use of medicinal plants, due to various factors such as increasing reliance on modern healthcare practices and a lack of exposure to traditional medicinal knowledge.

**Age groups.** Our investigation revealed that elderly population holds the majority of traditional medicinal knowledge, which has been passed down to them orally through generations. Among the different age groups, oldest participants (> 60 years) were the most frequent users of medicinal plants, with a rate of 34.24% (as depicted in figure 4). The usage rates for the other age groups were as follows: 26.03% for individuals aged 51 to 60, 20.55% for ages 41 to 50, 15.07% for ages 31 to 40, 2.74% for ages 20 to 30, and 1.37% for those under 20. The lower usage rate among the youngest age group can be attributed to their relative lack of chronic pathologies, such as diabetes. The significant experience of older individuals in employing medicinal plants is evident, highlighting the vital role of intergenerational knowledge transfer in preserving traditional medical practices.

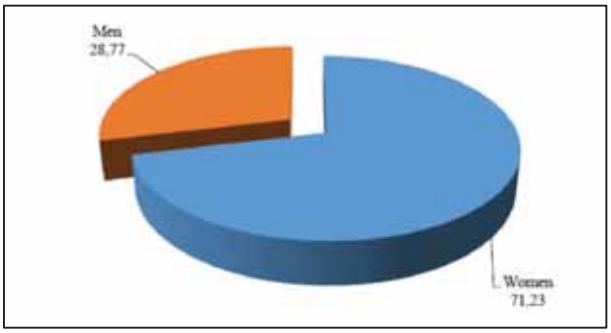



**FIGURE 2**
**Distribution of users by gender**

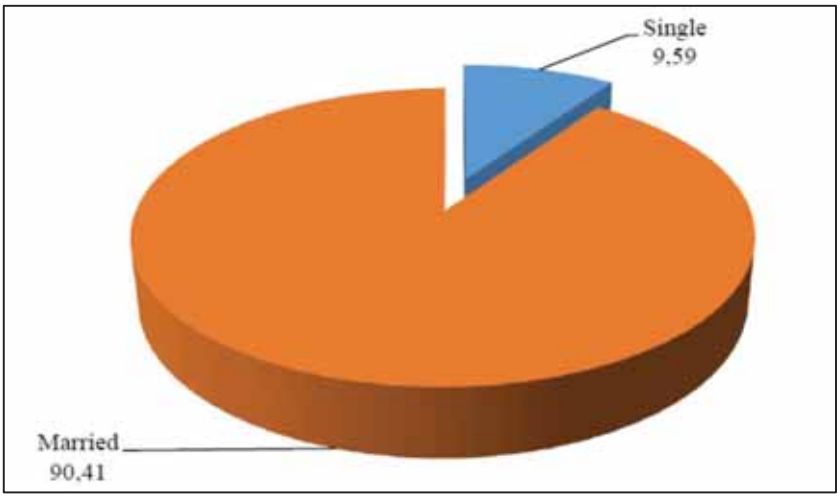



**FIGURE 3**
**Distribution of users by family situation**

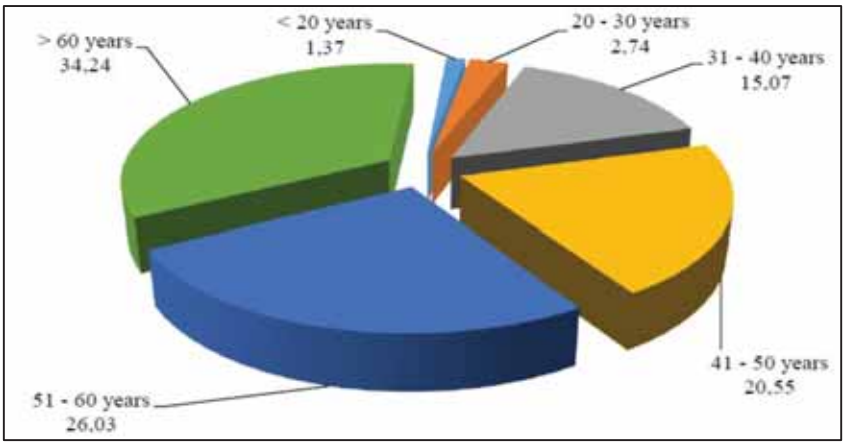



**FIGURE 4**
**Distribution of users by age group**

**Intellectual level.** The survey results demonstrate that the primary users of medicinal plants were individuals with limited education, as 41.49% of illiterate respondents reported using medicinal plants (figure 5). Furthermore, a considerable percentage of users with primary (24.66%) and middle school (19.18%) education also employ phytotherapy.

However, the propotion of users with higher education levels (secondary and university) was relatively lower, with the rate of 9.59% and 5.08% respectively. This results can be attributed to their perception that plants might pose risks and lead to multiple complications, particularly in cases of chronic pathologies whether used irritationally. Hence, they tend to be more cautious and sceptical about traditional medicinal practices involving plants. Our findings were consistent with previous research, [27, 25, 28, 26, 24] which similarly reported that illiterate individuals were the predominant users of medicinal plants.

**Floristic analysis. Analysis of botanical families.** The analysis of botanical families based on the identified plant species revealed a total of 29 botanical families, comprising 46 genera and 50 species of medicinal plants used in Sidi Bel Abbes region for treating diabetes (figure 6). Lauraceae family dominated, consisting of 8 species, which accounts for 16% of the total medicinal plant species identified. Following closely, Asteraceae represented 10% with 5 species, while Fabaceae and Rosaceae each contributed 6% with 3 species in each family.

Besides, the following botanical families: Amaranthaceae, Apiaceae, Cucurbitaceae, Myrtaceae, Oleaceae, and Zingiberaceae, each possessed 2 species, amounting to 4% of the identified medicinal plants. The remaining botanical families, including Amaryllidaceae, Anacardiaceae, Apocynaceae, Araliaceae, Asphodelaceae, Berberidaceae, Burseraceae, Cupressaceae, Ephedraceae, Gentianaceae, Moraceae, Plantaginaceae, Poaceae, Punicaceae, Ranunculaceae, Rutaceae, Sapindaceae, Urticaceae, and Zygophylaceae, were sparsely represented, each having a single species, accounting for 2% of the identified medicinal plants in the region.

**Ethnobotanical analysis. Parts used.** The analysis of the collected information highlighted that the leaf was the most frequently utilized plant part in the preparation of various medicinal remedies, accounting for 49.26% of all plant parts used (figure 7). It was evident that the leaf represented nearly half of the plant material used for therapeutic purposes. Following the leaf, other plant parts used in descending order were the stem (13.43%), seeds and flowers (7.46%), fruits (5.97%), bark (4.48%) and roots and rhizomes (2.99%). Collectively, these parts account for a significant portion of the plant material employed in conventional medicine.

In contrast, other plants organs such Pulp, bulb, resin and clove, were less commonly utilized, constituting only 1.49% of the overall usage.

The observed results were consistent with findings from several other studies [27, 25, 29, 30, 28, 26, 31], all of which concurred that leaves were the most widely used plant organs in traditional medicinal practices. This further validates the prominence of leaves in traditional remedies and the relevance of the study's findings within the broader context of ethnobotanical research.

The preference for using leaves in traditional medicine can be attributed to several factors. Firstly, leaves are readily accessible, and their harvesting is relatively straightforward and quick. Secondly, leaves are essential sites for photosynthesis, biosynthesis, and the accumulation of numerous secondary metabolites known for their pharmacological properties. As a result, leaves often contain a rich concentration of medical compounds, making them valuable resources for therapeutic use.

**Methods of preparation.** Our analysis reveals that decoction was the most commonly used method of preparation, accounting for 49.21% of the cases (figure 8). It was followed, in descending order, by infusion (30.16%), powder (9.52%), consumption in the raw state (4.62%), uncooking and poultice (4.76%), and lastly, the use of oil (1.59%), which is the least frequent mode.

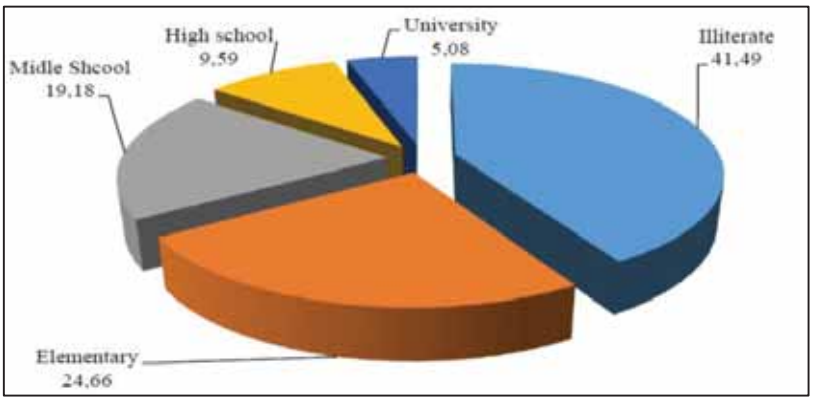



**FIGURE 5**
**Distribution of users by intellectual level**

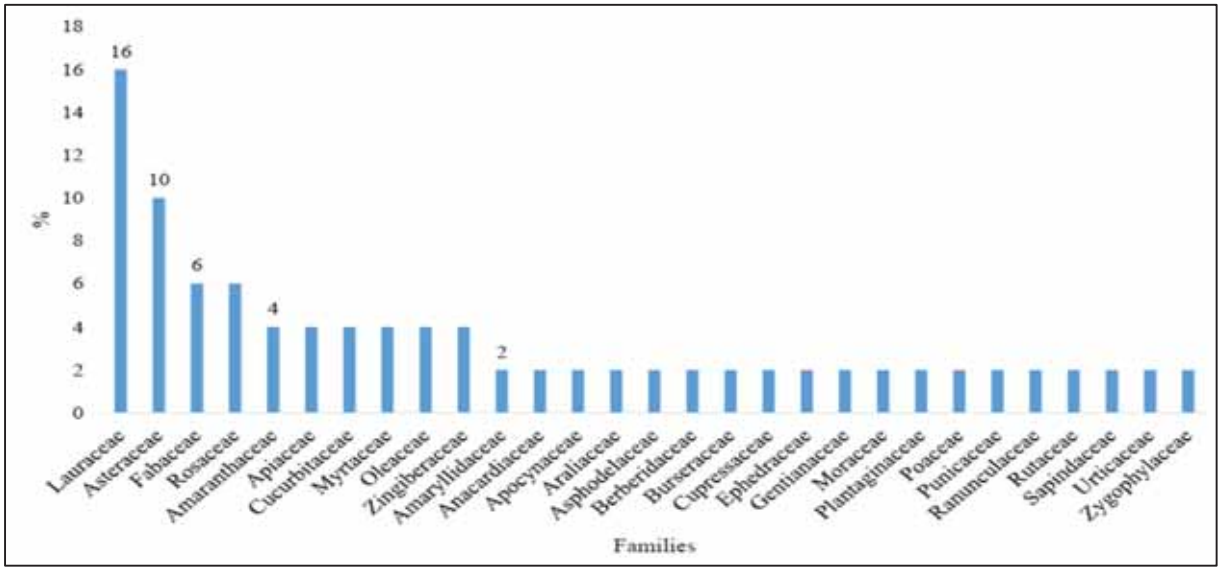



**FIGURE 6**
**Contribution of botanical families in the inventory**

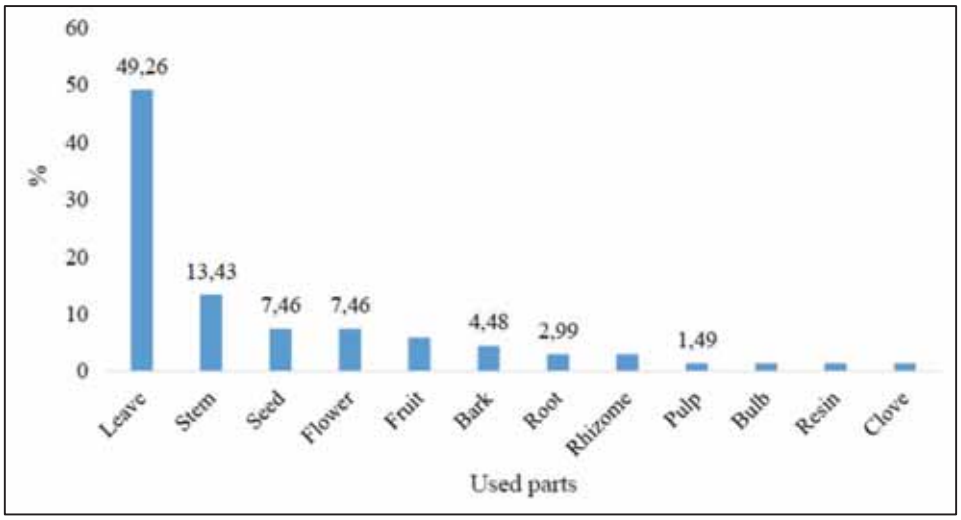



**FIGURE 7**
**Used parts**

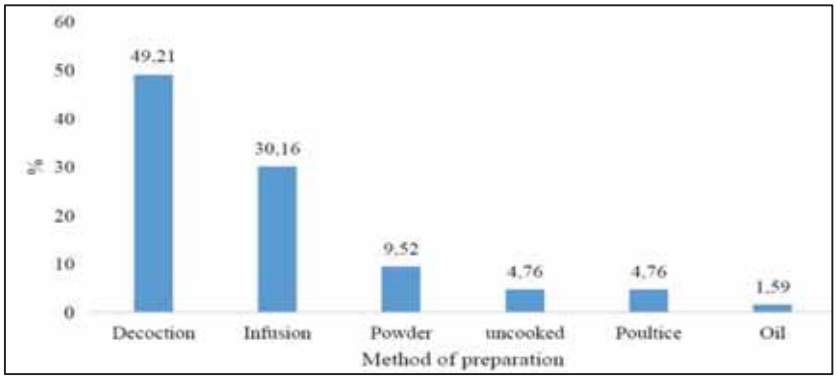



**FIGURE 8**
**Methods of preparation**

The preference for decoction can be attributed to its ability to quickly extract and assimilate the active ingredients from the medicinal plants, as it has a warming impact on the body. However, it is worth noting that this method may result in the destruction of certain compounds that are sensitive to high temperatures.

On the other hand, infusion was favored by users due to its ability to preserve the therapeutic properties of the plants, making it a popular choice for preparation.

These findings were consistent with other research studies [25, 30, 26, 24], which similarly showed that decoction is the most recommended method of preparation in traditional medicinal practices.

**Citation index.** The phytotherapeutic citation index (PCI) is a valuable metric used to analyze and compare the frequency of citation or usage of different plant species in the collected data [30]. By calculating the PCI for each plant, it becomes evident that certain species were cited or used more frequently than others in traditional medicinal practices.

According to the citation index, which indicates the frequency of use of each plants, the most used species were:

*Olea europeae* var. *sativa* L. (olive tree) (PCI=5.89%) enhances the release of insulin triggered by glucose peak during meals, thus enabling better cellular absorption of glucose. It induced an increase in glucose utilisation, leading to a secondary decrease in blood glucose levels [33].

*Trigonella foenum-graecum* L. (Fenugreek) (PCI=5.01%) leads to an increase in hepatic glycogen, stimulates glucose transport into adipocytes, and reduces carbohydrate digestion [34]. The compound 4-hydroxyisoleucine indues insulin release by directly affecting isolated langerhans islets in both rats and humans. It also inhibits intestinal glucose absorption by inhibiting alpha-glucosidase enzymes [35].

The extract of *Teucrium polium* L. (PCI =4.37 %) also has an effect on the regeneration of β-cells, which normalizes insulin release and peripheral metabolism, leading to a reduction in blood glucose levels. Several studies have demonstrated that extracts from this plants possess anti- α-amylase efficacy [36].

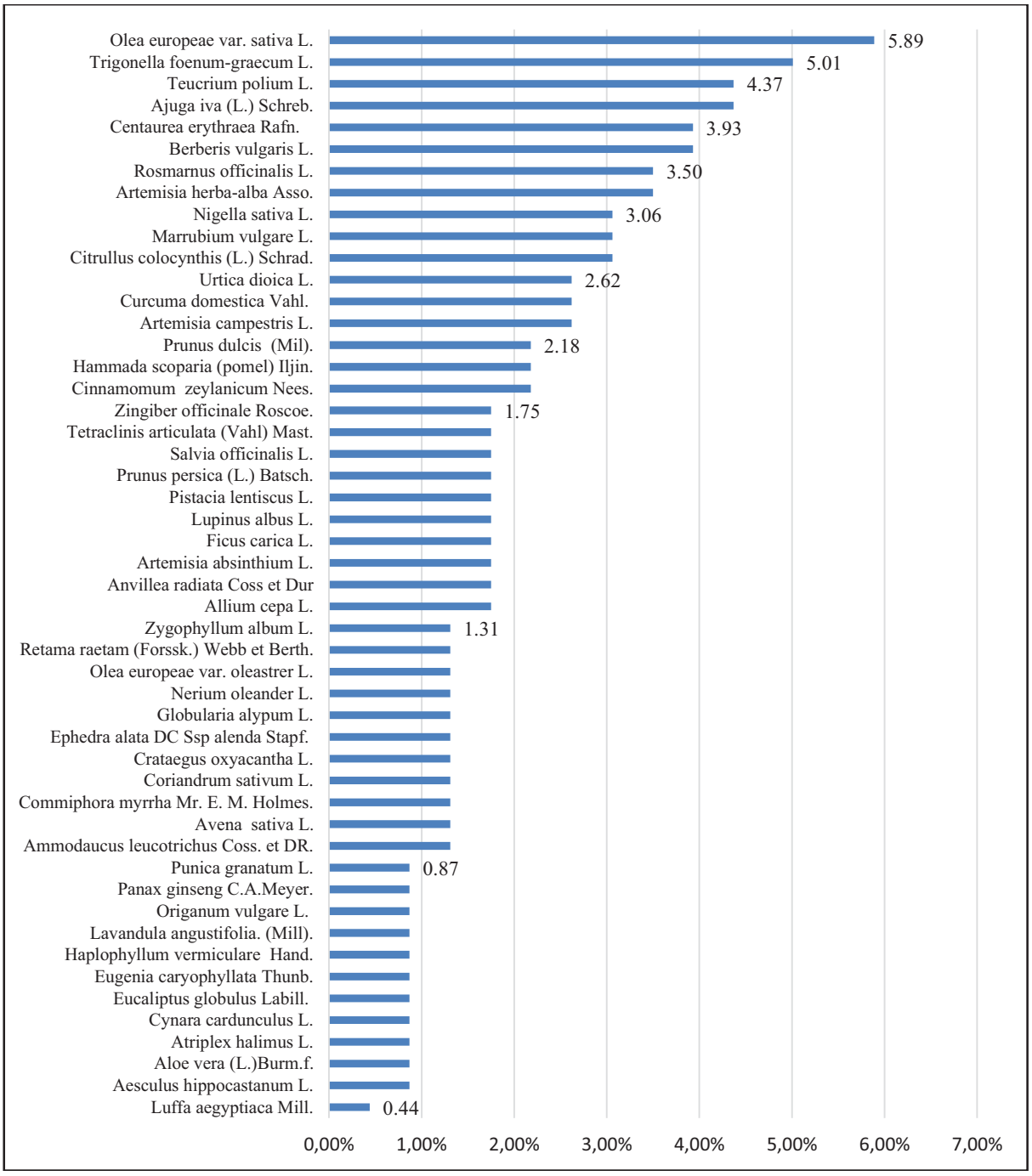



**FIGURE 9**
**Phytotherapeutic citation index**

The extract of *Ajuga iva* (L.) Schreb. (PCI=4.37%) has a strong hypoglycemic effect. The administration of the extract causes a reduction in blood glucose levels after 6 hours in diabetic rats. It significantely decrease glucose levels without any modification of plasma insulin concentration [37].

The extract *Centaurea erythraea* Rafn. (ICP=3.93 %) inhibits the enzymatic activity of α-amylase and α-glucosidase. This effect can delay the degradation of starch and oligosaccharides, which may lead to a decrease in glucose absorption and an elevation in postprandial blood glucose levels [38].

*Berberis vulgaris* L. (ICP=3.93 %) exhibits remarkable antidiabetic activity [39, 40].

Through its hypoglycemic effect, *Rosmarnus officinalis* L. (PCI=3.50 %) leads to an endogenous inhibition of glucose production [41]. It also inhibits intestinal glucose absorption [42] and α-glucosidase [43].

*Artemisia herba-alba* Asso. (PCI=3.50 %) plays significant role in the prevention of insulin resistance [44, 45].

*Nigella sativa* L. (PCI=3.06 %) inhibits neoglucogenesis. It further improves the cellular and subcellular structure of pancreatic β-cells [46].

*Marrubium vulgare* L. (PCI=3.06 %) exhibits antidiabetic activity due to the abundance of its aqueous extract in compounds known for their antidiabetic activities (flavonoids and verbascosides). The mechanism of action could involve the stimulation of insulin secretion by beta cells in the islets and/or the inhibition of insulin degradation process [47].

The powder of *Citrullus colocynthis* (L.) Schrad. (PCI=3.06 %) has an antidiabetic effect on type 2 diabetic patients, and the ß-(Pyrazol-1-yl)-L-alanine present in the seeds has an insulin-stimulating activity [48]. This plant exhibits insulinotropic activity and stimulates its secretion [49].

## CONCLUSION

Diabetes remains a highly prevalent and alarmingly increasing systemic disease, thus representing a serious public health issue. The state bears enormous expenses for the management of diabetic patients, particularly in developing countries.

The use of medicinal plants is strongly prevalent among diabetic patients, serving as a complement and an alternative to synthetic medications. The diversity of plants employed to treat diabetes, as well as their frequency of mention, reaffirms the significant role of medicinal plants in the health care system of the indigenous population of Sidi Bel Abbes. The conducted ethnobotanical study demonstrated that traditional medicine remains a widespread practice and enjoyed extensive utilization among the local population of Sidi Bel Abbes region.

Indeed, 50 species known for their hypoglycemic properties and used in the preparation of traditional remedies have been identified. These species belong to 29 different families, with Lauraceae and Asteraceae being the most commonly used ones.

The most frequently used species, listed in descending order of their phytotherapeutic citation index, were *Olea europeae* var. *sativa* L., *Trigonella foenum-graecum* L., *Teucrium polium* L., *Ajuga iva* (L.) Schreb., *Centaurea erythraea* Rafn., *Berberis vulgaris* L., *Artemisia herba-alba* Asso., *Nigella sativa* L., *Marrubium vulgare* L., *Citrullus colocynthis* (L.) Schrad.

The frequency of using hypoglycemic plants in the studied region was strongly linked to the profile of the surveyed individuals. Therefore, both female and male share medicinal knowledge, however there was a clear dominance of female (71,23 %). Married individuals hold the traditional therapeutic information, primarily due to their role as mothers (90,41 %). Illiterate individuals show a higher attraction towards herbal medicine.

From an ethnobotanical perspective, leaves were the main plant part used (48,33 %) due to their richness in secondary metabolites, and the most common form of preparation was decoction, as it provides warmth and allows for rapid extraction of active principles.

**TABLE 2**
**Classification of plants used for diabetes (vernacular names, citation index, utilized parts, and preparation methods)**

| No | Scientific name | Common name | Used parts | Method of preparation | PCI |
|---|---|---|---|---|---|
| **1** | *Luffa aegyptiaca* Mill. | Hebayelt el hammam | Fruits | Poultice | 0,44% |
| **2** | *Aesculus hippocastanum* L. | Kastal hindi | Seeds | Uncooked | 0,87% |
| **3** | *Aloe vera (L.)Burm.* | Sabar | Leaves | Infusion Decoction | 0,87% |
| **4** | *Atriplex halimus* L. | Gatfa | Leaves | Decoction | 0,87% |
| **5** | *Cynara cardunculus* L. | Khorchef | Leaves Stem | Decoction | 0,87% |
| **6** | *Eucaliptus globulus* Labill. | Kalitus | Leaves | Decoction | 0,87% |
| **7** | *Eugenia caryophyllata* Thunb. | Qronfel | Cloves | Decoction | 0,87% |
| **8** | *Haplophyllum vermiculare* Hand. | El fijel | Leaves | Decoction | 0,87% |
| **9** | *Lavandula angustifolia.* (Mill). | Khezama | Flowers | Infusion | 0,87% |
| **10** | *Origanum vulgare* L. | Zaatar | Aerial parts | Infusion Decoction | 0,87% |
| **11** | *Panax ginseng* C.A.Meyer. | ginsingh | Roots | Decoction | 0,87% |
| **12** | *Punica granatum* L. | Romman | Fruit peel | Decoction | 0,87% |
| **13** | *Ammodaucus leucotrichus* Coss. et DR. | Kamoun essofi | Fruits | Decoction | 1,31% |
| **14** | *Avena sativa* L. | khortane | Seeds | Decoction | 1,31% |
| **15** | *Commiphora myrrha* Mr. E. M. Holmes. | El morra | Resin | Decoction | 1,31% |
| **16** | *Coriandrum sativum* L. | Qezbor | Aerial parts Seeds | Uncooked Infusion Powder | 1,31% |

| | | | | | |
|---|---|---|---|---|---|
| **17** | *Crataegus oxyacantha* L. | Zaârour, Baba adjina | Leaves | Infusion | 1,31% |
| **18** | *Ephedra alata* DC Ssp *alenda* Stapf. | Alenda Adam | Stem | Decoction | 1,31% |
| **19** | *Globularia alypum* L. | Tasselgha, Aïn larnab | Leaves | Infusion | 1,31% |
| **20** | *Nerium oleander* L. | ddeflâ | Leaves | Decoction Poultice | 1,31% |
| **21** | *Olea europeae var. oleastrer L.* | Zebbouj | Leaves | Decoction | 1,31% |
| **22** | *Retama raetam* (Forssk.) Webb et Berth. | Remth lahmar | Leaves | Decoction | 1,31% |
| **23** | *Zygophyllum album* L. | Aggaya | Leaves Stem | Infusion Decoction | 1,31% |
| **24** | *Allium cepa* L. | Elbasla | Bulb | Uncooked Infusion | 1,75% |
| **25** | *Anvillea radiata* Coss et Dur | Negd | Leaves | Decoction | 1,75% |
| **26** | *Artemisia absinthium* L. | Chehiba | Stem Leaves | Infusion | 1,75% |
| **27** | *Ficus carica* L. | Karmouss | Leaves Fruits | Decoction Uncooked | 1,75% |
| **28** | *Lupinus albus* L. | Termas mur | Seeds | Infusion Powder | 1,75% |
| **29** | *Pistacia lentiscus* L. | Daroue | Leaves | Decoction | 1,75% |
| **30** | *Prunus persica (L.)* Batsch. | Khoukhe | Leaves | Decoction | 1,75% |
| **31** | *Salvia officinalis* L. | Miramiya, salmiya | Leaves | Infusion | 1,75% |
| **32** | *Tetraclinis articulata* (Vahl) Mast. | Araâr | Aerial parts | Infusion | 1,75% |
| **33** | *Zingiber officinale* Roscoe. | Skin jebiir, Zenjabil | Rhizome | Decoction | 1,75% |
| **34** | *Cinnamomum zeylanicum* Nees. | Karfa | Stem bark | Decoction | 2,18% |
| **35** | *Hammada scoparia* (pomel) Iljin. | Remth | Leaves Stem | Decoction | 2,18% |
| **36** | *Prunus dulcis* (Mil). | Lûz mûr | Leaves Seeds | Decoction Infusion Powder | 2,18% |
| **37** | *Artemisia campestris* L. | Allal | Leaves | Infusion | 2,62% |
| **38** | *Curcuma domestica* Vahl. | Curcum | Rhizome | Decoction | 2,62% |
| **39** | *Urtica dioica* L. | Horaig | Leaves | Infusion | 2,62% |
| **40** | *Citrullus colocynthis* (L.) Schrad. | Lhdej | Pulp | Decoction Poultice | 3,06% |
| **41** | *Marrubium vulgare* L. | Marriwa | Leaves | Infusion | 3,06% |
| **42** | *Nigella sativa* L. | Haba sawda | Seeds | Decoction Powder | 3,06% |
| **43** | *Artemisia herba-alba* Asso. | Chih | Aerial parts | Infusion | 3,50% |
| **44** | *Rosmarnus officinalis* L. | Halhal, yazir | Leaves | Infusion | 3,50% |
| **45** | *Berberis vulgaris L.* | Ghris | Root bark Leaves | Decoction Powder | 3,93% |
| **46** | *Centaurea erythraea* Rafn. | Merrâret lehnech | Leaves | Infusion | 3,93% |
| **47** | *Ajuga iva* (L.) Schreb. | Chendgoura | Leaves | Infusion | 4,37% |
| **48** | *Teucrium polium* L. | Eljaeda | Leaves | Infusion | 4,37% |
| **49** | *Trigonella foenum-graecum* L. | Helba | Seeds Leaves | Decoction Powder Infusion | 5,01% |
| **50** | *Olea europeae* var. *sativa* L. | Zitoune | Leaves Fruits | Decoction Oil | 5,89% |

## REFERENCES


[1] World Health Organization (WHO) (2002) Programme on Traditional Medicine. (2002). WHO traditional medicine strategy 2002-2005. WHO/EDM/TRM/2002.1. Geneva, Suisse. 1-61 (Avaailable: https://apps.who.int/iris/bitstream/handle/10665/67313/WHO_EDM_TRM_2002.1_fre.pdf?sequence=1&isAllowed=y). (Accessed date: 14/05/2023) (in French)

[2] Dutertre, J.M. (2011) Prospectve survey a,ong the population consulting general practioners'offices on the island of Reunion : Regarding medicinal plants, usage, effects, safety, and relationship with the general practitioner. Thesis State PhD. Victor Segalen University, Bordeaux 2, Faculty of medical sciences, Bordeaux, France (in French).

[3] Pelt, J.M. (2001) The new naturel remedies. Edition Fayard, Paris, France. 1-324 (in French).

[4] Salemi, O. (2010) Food practices of diabetics: Study of several cases in Oran (Algeria), Économie Rurale. 318-319, 80-95 (in French).

[5] Makhlouf, M., Montaigne, E. and Tessa, A. (2015) Algerian dairy policy : between food security and differential support for consumption. New Medit. 14(1), 12-23 (in French).

[6] Benabdeli, K. (2000) Assessment of the impact of new livestock farming methods on the steppe environment and space: case of Ras El Ma municipality (Sidi Bel Abbes, Algeria). Options Méditerranéennes A. 39, 129-41 (in French).

[7] Quézel, P., Santa, S. (1962) New flora of Algeria and southern regions. National Scientific Research Center (NSRC) Edition, Volume 1, Paris, France. 1-570 (in French).

[8] Quézel, P., Santa, S. (1963) New flora of Algeria and southern regions. National Scientific Research Center (NSRC) Edition, Volume 2, Paris, France.1-517 (in French).

[9] Beauvais, M. (2000) Medicinal plants. Gründ Edition, Beaumont, France. 1-95 (French).

[10] Beloued, A. (2001) Medicinal plants of Algeria, University Publications Office (OPU) Edition, Algiers, Algeria. 1-284 (in French).

[11] Teuscher, E., Anton, R., Lobstein, A. (2005) Aromatic plants. Lavoisier Edition, Paris, France. 1-522 (in French).

[12] Debaisieux, F., Polese, J.M. (2009) Medicinal plants : Secret and remedies of the past. Debaisieux Edition, Beaumont, France. 1-127 (in French).

[13] Salhi, S., Fadli, M., Zidane, L., Douira, A. (2010) Floristic and ethnobotanical studies of medicinal plants in the city of Kénitra (Morocco). Lazaroa. 31, 133-146 (in French).

[14] Fadi, Z. (2011) Rosemary *(Rosmarinus officinalis)* The proper extraction process for optimal therapeutic effect. Doctoral thesis in pharmacy. Mohammed V University, Faculty of Medicine and Pharmacy, Rabat, Morocco (in French).

[15] Hadj Moussa, A. (2012) Contribution to in vitro study of the effect of *Retama retam* leaf extract on α-amylase activity. Higher diploma in chemestry, Abou Bekr Belkaid University, Tlemcen, Algeria (in French).

[16] Chevallier, A. (2013) Medicinal plants. Gründ Edition, Beaumont, France. 1-287 (in French).

[17] Benkhnigue, O., Ben Akka, F., Salhi, S., Fadli, M., Douira, A., Zidane, L. (2014) Catalogue of medicinal plantsbused in the treatment of diabetes in the d'Al Haouz-Rhamna region (Marocco). Journal of animal and plant sciences. 23(1), 3539-3568 (in French).

[18] Ait Ouakrouch, I. (2015) Ethnobotanical survey regarding medicinal plants used in the traditional treatment of type II diabetes in Merrakech. Doctorate thesis in medicine. Faculty of Medicine and Pharmacy, Marrakech, Morocco (in French).

[19] Saidi, A., Ali Belhadj, O. (2016) Survey on antihypertensive plants in the Tlemcen region. Doctor of pharmacy thesis, Abou bekr Belkaid University, Faculty of Medicine, Tlemcen, Algeria (in French).

[20] Boumediou, A., Addoun, S. (2017) Ethnobotanical study on the use of toxic plants in traditional medicine in the city of Tlemcen (Algeria). Doctor of pharmacy thesis, Abou bekr Belkaid University, Faculty of Medicine, Tlemcen, Algeria (in French).

[21] Dusser Lauge, N. (2017) Study of the medicinal plants in the Maghreb: Traditional uses and phytochemical studies. Pharmacy doctoral thesis, Paul Sabatier University, Toulouse 3, France (in French).

[22] Palici, I.F. (2017) Valorization of biological activities of several north African Saharan plant species. Pharmacy doctoral thesis, Victor Segalen University, Bordeaux 2, Faculty of medical sciences, Bordeaux, France (in French).

[23] Tachema, A., Bendimerad, S. (2018) Survey on the usage of medicinal plants by female patients with breast cancer at the university Hospital Center (UHC) of Tlemcen. Doctor of pharmacy thesis, Abou bekr Belkaid University, Faculty of Medicine, Tlemcen, Algeria (in French).

[24] Bouzid, A., Chadli, R., Bouzid, K. (2017) Ethnobotanical study of the medicinal plant Arbutus unedo L. in the region of Sidi BeL abbes. Phytotherapie. 15, 373-378 (in French).

[25] Lahsissene, H., Kahouadji, A. (2010) Ethnobotanical study of medicinal and aromatic plants in the Zaër region of Morocco. Phytotherapie. 8(4), 210-217 (in French).

[26] Daoudi, A., Bammou, M., Zarkani, S., Slimani, I., Ibijbijen, J., Nassiri, L. (2016) Ethnobotanical study of medicinal flora in rural municipality of Aguelmouss - Khenifra province – (Morocco). Phytotherapie. 14(4), 220-8 (in French).

[27] Mehdioui, R., Kahouadji, A. (2007) Ethnobotanical study among the population living near the Amsittène forest: A case study of Imi n'Tlit municipality (Essaouira province. Bulletin de l'Institut Scientifique de Rabat. 29, 11-20 (in French).

[28] Adouane, S. (2016) Ethnobotanical study of medicinal plants in the southern Aurès region. Master's thesis, Mohamed Khider University, Biskra, Algeria (in French).

[29] Salhi, S., Fadli, M., Zidane, L., Douira, A. (2010) Floristic and ethnobotanical study of medicinal plants in the city of Kénitra (Marocco). Lazaroa. 31, 133-46 (in French).

[30] Bahassan, A., Zidane, L., El Yacoubi, H., Rochdi, A. (2014) Ethnobotany and taxonomy of medicinal plants used for diseases treatment of the digestive system in Hadramaout (Yemen). Phytotherapie. 12(6), 399-416 (in French).

[31] Mikou, K., Rachiq, S., Jarrar Oulidi, A. (2016) Ethnobotanical survey of medicinal and aromatic plants used by the people of Fez in Morocco. Phytotherapie. 14(1), 35-43 (in French).

[32] Moatir, R., Fauron, R., Donadieu, Y. (1983) Phytotherapy : A distinct therapautic approach. Maloine Edition, Paris, France, 1-245 (in French).

[33] Gonzalez, M., Zarzuelo, A., Gamez, M.J., Utrilla, M.P., Jimenez, J., Osuna, I. (1992) Hypoglycemic activity of Olive leaf. Planta Medica. 58(6), 513-515.

[34] Hannan, J.M.A., Ali, L., Khaleque, J., Akhter, M., Flatt, P.R., Abdel Wahab, Y.H.A. (2007) Soluble dietary fibre fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. British Journal of Nutrition. 97, 514-521.

[35] Sauvaire, Y., Petit, P., Broca, C., Manteghetti, M., Baissac, Y., Fernandez-Alvarez, J., Gross, R., Roye, M., Leconte, A., Gomis, R., Ribes, G. (1998) 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. Diabetes. 47, 206-210.

[36] Asghari, A.A., Mokhtari-Zaer, A., Niazmand, S., Mc Entee, K., Mahmoudabady, M. (2020) Anti-diabetic properties and bioactive compounds of *Teucrium polium* L. Asian Pacific Journal of Tropical Biomedicine. 10(10), 433-441.

[37] Elhilaly, J., Lyoussi, B. (2002) Hypoglycaemic effect of the lyophilised aqueous extract of *Ajuga iva* in normal and streptozotocin diabetic rats. Journal of Ethnopharmacology. 80(2-3), 109-113.

[38] Loizzo, M., Saab, A., Tundis, R., Menichini, F. (2008) *In vitro* inhibitory activities of plants used in Lebanon traditional medicine against angiotensin converting enzyme (ACE) and digestive enzymes related to diabetes. Journal of Ethnopharmacology. 119, 109-116.

[39] Didarloo, A., Shojaeizadeh, D., Eftekhar Ardebili, H., Niknami, S., Hajizadeh, E., Alizadeh, M. (2011) Assessment of factors affecting self-care behavior among women with type 2 diabetes in Khoy City Diabetes Clinic using the extended theory of reasoned action. Journal of School of Public Health and Institute of Public Health Research. 9(2), 79-92.

[40] Meliani, N., Dib, M.E.A., Allali, H., Tabti, B. (2011) Hypoglycaemic effect of Berberis vulgaris in normal and streptozotocin-induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 1(6), 468-471.

[41] Eddouks, M., Jouad, H., Maghrani, M., Lemhadri, A., Burcelin, R. (2003) Inhibition of endogenous glucose production accounts for hypoglycemic effect of *Spergularia purpurea* in streptozotocin mice. Phytomedicine. 10, 594–599.

[42] Platel, K, Srinivasan, K. (1997) Plant foods in the management of diabetes mellitus: vegetables aspotential hypoglycaemic agents. Nahrung. 41(2), 68-74.

[43] Koga, K., Shibata, H., Yoshino, K., Nomoto, K. (2006) Effects of 50% Ethanol Extract from Rosemary (*Rosmarinus officinalis*) on Alpha-Glucosidase Inhibitory Activity and the Elevation of Plasma Glucose Level in Rats, and Its Active Compound. Journal of Food Science. 71(7), 507-512.

[44] Hamza, N., Berke, B., Cheze, C., Agli, A.N., Robinson, P., Gin, H., Moore, N. (2010) Prevention of type 2 diabetes induced by high fat diet in the c57bl/6j mouse by two medicinal plants used in traditional treatment of diabetes in the east of Algeria. Journal of Ethnopharmacology. 128(2), 513-518.

[45] Awad, N.E., Seida, A.A., Shaffie, Z.E.K.N., Abd El-Aziz, A.M. (2011) Hypoglycemic activity of Artemisia herba-alba (asso.) Used in egyptian traditional medicine as hypoglycemic remedy. Journal of Applied Pharmaceutical Sciences. 2(3), 30-39.

[46] Abdelmeguid, N.E., Fakhoury, R., Kamal, S.M., Al Wafai, R.J. (2010) Effects of *Nigella sativa* and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats. Journal of Diabetes. 2(4), 256-266.

[47] Jarald, E., Balakrishnan, J.S., Chandra D.J. (2008) Diabetes and herbal medicines. Iranian Journal of Pharmacology and Therapeutics. 7, 97-106.
[48] Benariba, N., Djaziri, R., Zerriouth, B.H., Boucherit, K., Louchami, K., Sener, A., Malaisse, W.J. (2009) Antihyperglycemic effect of *Citrullus colocynthis* seed aqueous extracts in streptozotocininduced diabetic rats. Metab Func Res Diabetes. 2, 71-76.
[49] Shahzadi, I., Fazal, R.M., Hussain, Z., Yasin, R., Aziz, M.K., Samiullah, K., Malik, I.U., Waseem, M., Yar, A., Khan, S.H., Ilyas, M. (2022) Anti-diabetic Effects of Aqueous plant (Citrullus colocynthis) Extract in Streptozotocin Induced Diabetic Mice. Haya Saudi Journal of Life Sciences. 7(6), 199-205.

**Received:** **03.08.2023**
**Accepted:** **12.08.2023**

**CORRESPONDING AUTHOR**

**Nadjia Fertout-Mouri**
Laboratory of Plant Biodiversity: Conservation and Valorization
Djillali Liabès University of Sidi Bel Abbes
Algeria

e-mail: nadjiafertout@yahoo.fr

-© by PSP Volume 32– No. 09/2023 pages 3019-3027 Fresenius Environmental Bulletin

# PREPARATION OF A NOVEL HAMMADA SCOPARIA OINTMENT AND ASSESSMENT OF ITS IMPACT ON WISTAR RAT CUTANEOUS WOUND HEALING

**Ibtissam Gheffari[1,*], Abdelkrim Kefifa[1], Khaled Kahloula[1], Kheira Rebbah[2], Ahmed Abdelhammid Djebbar[3], Djehina Brinissat[4], Samira Meziani[2], Abbassia Demmouche[2], Lahouaria Labga[2]**

[1]Laboratory of Biotoxicology, Pharmacognosy and Biological Valorization of Plants, Department of Biology, University of Saida Dr- Moulay Taher, 20 000 Saida, Algeria
[2]Biotoxicology Laboratory, Department of Biology, Faculty of Natural and Life Sciences, Djillali Liabes University, Sidi-Bel-Abbes, Algeria
[3]Environment and Health Research Laboratory, University Djilali Liabes, Sidi-Bel-Abbes, Algeria
[4]Applied Microbiology Laboratory, Department of Biology, Faculty of Natural and Life Sciences, Djillali Liabes University, Sidi-Bel-Abbes, Algeria

## ABSTRACT

The aim of this endeavor was to make preparations for an ointment from aqueous extract from Hammada scoparia. two formulations with different percentages of Hammada scoparia: 10% and 3% were prepared. The antioxidant activity of lyophilisate was established in vitro and assessed in vivo by observing the process of wound healing. Data revealed that Hammada scoparia formulation showed the most substantial antioxidant potency in vitro and after twelve days of therapy, dramatically accelerated wound healing, as evidenced by a greater wound percentage of areas closure (98%) confirmed by histological analysis, in comparison to the control, Mebo, and pure vaseline groups. In general, these results showed that the 10% Hammada scoparia formulation had a greater capacity for wound healing, which was confirmed by its significant in vitro antioxidant activity.

**KEYWORDS:**
Ointment, Aqueous extract, Antioxidant activity, Wound healing

## INTRODUCTION

The complex organ known as the skin covers the whole surface of the body. It is the biggest organ in the body and makes up around 15% of the weight of an adult[1]. The primary role of the skin is to protect the homeostasis of the internal environment by separating it from the external environment, preventing water loss, and creating an essential barrier [2]. The term "wound" refers to any disruption of the anatomic continuity of a tissue or rupture in the cellular integrity of a tissue that is mostly caused by a chemical, microbiological, physical, or thermal insult [3-5]. Hemostasis is the first step in a complicated biological process known as wound healing, which is then followed by inflammation, proliferation, and remodeling[5, 6]

In most societies, the fundamental sources of medicines utilized for treating a variety of ailments are widely considered to be plants[7]. This has encouraged numerous scientists and pharmaceutical companies to focus on plant-based therapies.,

In recent years, wounds have developed into a very difficult pathological problem [3], [4], [5]. Due to their high occurrence and the high expense of managing chronic wound situations, wounds have a significant socioeconomic impact.

Hammada scoparia is a promising source of bioactive substances. Moreover, its potential has been showcased in manifesting antioxidant[8],[9], antimicrobial [9], and anti-inflammatory characteristics[10], [9] that play a substantial role in enhancing the wound healing process.

A medicinal plant called Hammada scoparia is a member of the Chenopodiaceae family, which comprises more than 1300 species and 120 genera[11], It is a tiny, densely branching shrub found in North Africa, Syria, Iraq, and portions of Iran, Turkey, and southeastern Spain [12]. This well-known plant is frequently employed to treat a variety of illnesses as decoction, infusion, or cataplasm.

Given this context, we undertook this study to evaluate a new formulation's potential for wound healing utilizing an in vivo method.

## MATERIALS AND METHODS

**Plant material.** The botanical material consists of the aerial part of Hammada scoparia collected on May 2022 from Cellala city (south-western Algeria) located 80 km from Aïn Sefra city (N 33° 01′ 59″, E 0° 03′ 20″ ) . The botanical identification was carried

out by the botanist professor "Hasnaoui O", in the Faculty of Science.

**Extract preparation method.** The constituents of Hammada scoparia aerial parts were cleansed, dried for seven days under room temperature in a shaded environment, then crushed into a fine powder, and preserved in sterile and humid-free conditions. After extracting the powdered aerial portions of HS (100 g) for 20 minutes with 1000 ml of boiling water, the cooked decoction was filtered through Whatman filter paper and lyophilized.

**Ointment preparation.** Vaseline served as the principle excipient because it was stable and compatible with the majority of the active principles; the lyophilisated material was then reconstituted with distillate water before being incorporated. Two ointments 3% and 10% in Vaseline were produced from the dry aqueous extract powder (lyophilisate) of the aerial part of Hammada scoparia. As a reference preservative, sodium benzoate was utilized at a rate of 1.5 g per 1000 g of ointment. In a mortar using a pestle, lyophilisate and sodium benzoate were triturated. The mixture was then reconstituted with distilled water before being incorporated into Vaseline with gentle stirring until homogenization. The ointments were stored in hermetically sealed jars and maintained at room temperature out of direct sunlight.

**Experimental study. Animals.** Twenty-five adult Wistar rats weighing between 190 and 210 g, obtained from the Pasteur Institute of Algeria. All rats were maintained in standard conditions with a temperature of 23°C ± 2°C and 12 h light-dark cycle, and they were allowed to free access of water and food. The animals were kept in individual cages.

The rats were weighed, put under anesthesia, and then excisions were performed on the rats' necks after shaving the animal and disinfecting the skin with alcohol. Using forceps and scissors, a 1 cm-diameter circle of skin was sliced (Figure 2). Ketamine hydrochloride 50 mg/ml was injected at a dose of 2 ml / kg of body weight, along with 5 mg/kg of diazepam, to provide anesthesia.

**Experimental Design.** 25 females were separated into five lots of five animals after the skin excision:

**control group:** rats received treatment with a physiological saline solution.

**Mebo group:** rats received treatment with a standard medication "Mebo" cream.

**3% formulation group:** rats received treatment with 3% Hammada scoparia plant.

**10% formulation group:** rats received treatment with 10% Hammada scoparia plant.

**Blank group:** rats received treatment with pure vaseline.

**Measurement of wound area.** All wounds were photographed digitally during the period of the healing process, and the wound area was manually traced. In order to design and draft, Autodesk Auto CAD was used to measure the wound surface areas. The following equation was used to determine the wound contraction rate: (Eq. 1)

**Histological examination.** Following rat sacrifice, tissue samples were extracted from the wound site from all rat groups. These samples were then immediately fixed in a solution of neutral-buffered formalin (10%), embedded in paraffin wax, sectioned to a thickness of 5 µm, and subjected to hematoxylin-eosin staining. A light microscope was then utilized to analyze and take photographs of these samples.

**Antioxidant activity.** Two techniques were employed to evaluate the antioxidant activity of Hammada scoparia aqueous extract. The first involves evaluating the extract's ability to inhibit the DPPH radical. The second test, called FRAP, evaluates the extract's capacity to reduce ferric ions (Fe3+).

**DPPH free radical scavenging test.** This test was carried out in accordance with the protocol provided by [13], the absorbance was assessed at 517 nm using a spectrophotometer, and the percent inhibition of the DPPH radical was calculated using the formula below: (Eq. 2)

**Ferric antioxidant reducing power (FRAP).** Utilizing an aqueous extract from Haloxylon scoparium, the reducing power of ferric ions (Fe3+) is determined using the method provided by Goncalves [14]. At 700 nm, the absorbance was spectrophotometrically measured. The reaction mixture's increased absorbance was interpreted as an increase in the extract's reducing activity, and the result was compared to ascorbic acid, which served as a positive control.

$$\text{Rate of wound contraction } (\%) = \frac{initial\ surface\ size - specific\ day\ surface\ size}{initial\ surface\ size} \times 100 \quad \text{Eq. 1}$$

$$(\%) d'inhibition = (\boldsymbol{A}\ blanc - \boldsymbol{A}\ échantillon / \boldsymbol{A}\ blanc) \times 100 \quad \text{Eq. 2}$$

**Statistical Analysis.** To identify differences within groups, a one-way analysis of variance (ANOVA) was utilized. All results are shown as mean ± S.E.M. If $p<0.05$, values were considered statistically significant. For the statistical analysis, the SPSS/PC program (Version 15.0; SPSS, Chicago, IL) was used.

## RESULTS

**Plant extract yield.** The quantity of the pulverized plant material used for the extraction was expressed as a percentage yield of the aqueous extract (HS), which was 12.64%.

**Antioxidant activities.** Figure 1 presents the results of this study as two straight lines, with the initial one illustrating the variation in DPPH percentage inhibition relative to ascorbic acid concentration. The process through which ferric ions (Fe3+) are reduced to ferrous ions (Fe2+) in the FRAP test is depicted by the second straight line as a function of ascorbic acid concentration.

**Morphological evaluation.** Following the use of a circular excision wound model, fourteen days of continuous observation were used to examine the wound healing processes in rats.

Daily applications of the ointment were made, and the colorimetric evaluation was used to measure how well the wounds were healing. The typical photographs captured on day 0, 6, 9, 12, and 14 are shown in Figure 2.

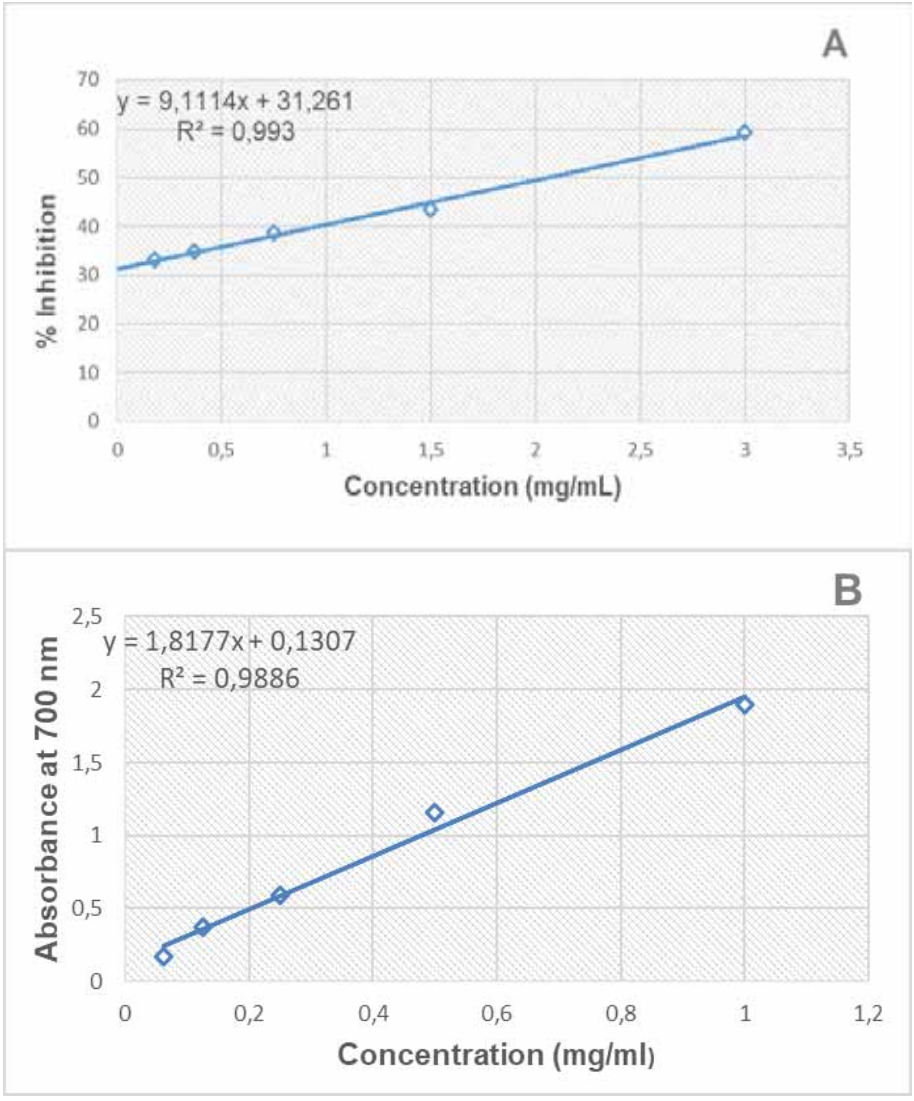



**FIGURE 1**
**Linear straight representing alterations in percent inhibition (A) and absorbance (B) in relation to different concentrations of ascorbic acid for the DPPH and FRAP reduction tests of Hammada Scoparia.**

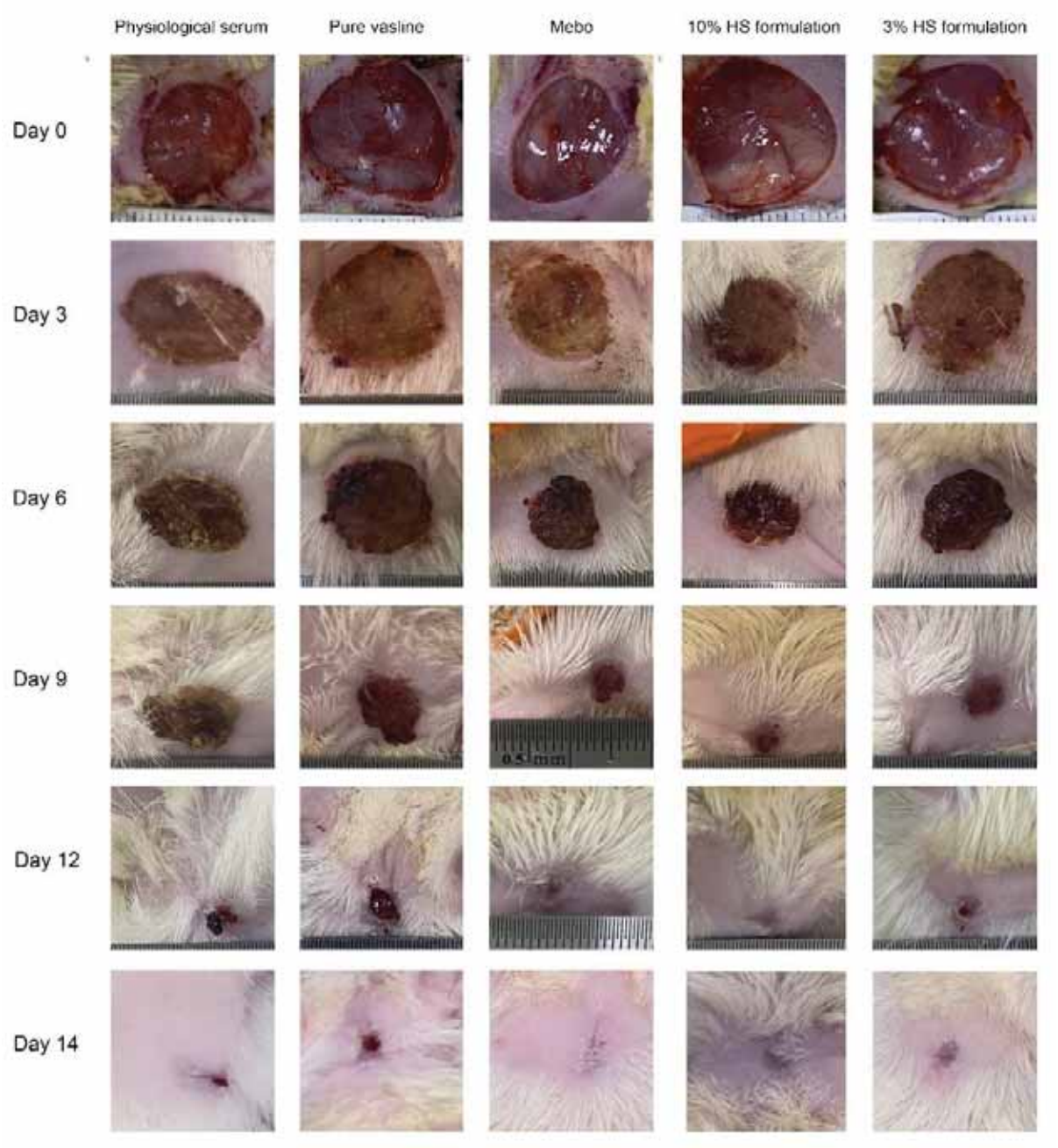



**FIGURE 2**
**Representative images of wound healing effects at days 0, 6, 9, 12, and 14 of the group treated with physiological serum, pure vaseline, Mebo, 10%, and 3% HS formulation.**

**Assessment of wound closure.** Every third day during the experimental period, the size of the regions was measured in order to assess the wound contraction. as depicted in Figure 3, the groups received 10% ointment plus the drug "Mebo" had superior rates of contraction than the control group, which received physiological serum as a treatment, and the group given pure vaseline. Beginning on the seventh day, rats given 10% ointment showed a much higher potential for healing than the other groups. The wound in the untreated group remained unhealed on the final day of the experiment.

**Histopathological examination.** Figure 4 shows histopathological micrographs of rats treated different formulations.

## DISCUSSION

Two tests were used to assess the lyophilized extract of the Hammada scoparia antioxidant capacities: DPPH free radical scavenging study, and ferric antioxidant reducing power (FRAP), As seen in Figure 3, the aqueous extract has significant antioxida activity ($p < 0.05$), with almost 59,2% ± 4.31 for

DPPH free radical scavenging activity, DO = 0.375± 0.079 for reducing power, and DO = 1,893±0,625 for iron (Fe2+) chelating activity, Several research has indicated a correlation between the outcomes of the two antioxidant activity assessments (DPPH and FRAP) in the literature.[15] These results agree with those achieved by Bouaziz and associates utilizing the Tunisian aqueous leaf extract of H. scoparia.[9] and those obtained by Lachkar from Morocco. [16]

These results highlight Hammada scoparia abundance in natural antioxidants, which might help to explain why traditional medicine practitioners are interested in using it. These chemicals' redox characteristics, which enable them to function as reducing agents and occasionally as hydrogen donors, are primarily responsible for their antioxidant effect. [17]

All wounds displayed a same brilliant red color on the day of zero. The untreated wound showed an increased inflammatory border surrounding the impaired skin starting on the SIXTH day of treatment. However, the treated groups (P10% and "Mebo") had a brown color as a result of the development of the crust, which indicated the beginning of the healing process via the creation of a blood clot.

After nine days, the wound area reduced and exposed tissue that was pinkish in color. This was seen in the treated groups. Additionally, untreated rats still exhibit an expanded inflammation with a dark red color (physiological serum and pure vaseline). The group that received our 10% ointment on day 12 observed that the sores had completely healed. However, the untreated and pure vaseline-treated groups still had open wound and scabs. These results highlighted 10% HS formulation healing powers and demonstrated how HS could potentially be used to quicken wound healing. These observations align with outcomes obtained by [18] and [1].

The 10% ointment-treated rats had complete size reduction in the wound site compared to the control group, and full restoration of the initial skin structures. Similar to this, the percentage of wound contraction promotes epithelization and cell proliferation, while simultaneously reducing the incidence of infections[19]. In contrast, the group treated with physiological serum displayed a slower healing process for an open wound compared to the other groups. According to these results, HS at 10% accelerates the repair of wounds, an essential phase in the healing process.

Almost completely complete wound contraction (98%),(91%) was seen in groups treated with formulations of 10% and Mebo on day 12 of the examination, contrary to the control, blank, and 3% treated groups. It is suggested that formulations created a microorganism-free environment for the skin to heal quickly after treatment as a result of their antioxidant and antibacterial properties [8],[9].

As previously mentioned, the process of cutaneous tissue healing is intricate and multifaceted. The histological assessment is a convincing method to support and evaluate wound healing, which can reveal information about the contents of collagen and fibroblasts, re-epithelization and neovascularization, and the deposition of inflammatory cells, collagen.[20]

Large numbers of macrophages, inflammatory cells, and dilated blood vessels were present in the control and blank groups [21]. In addition to excellent healing, wounds treated with Mebo also had a formed dermis and epidermis, which can be seen in the presence of neutrophils, dense fibrillar collagen matrix, and granulomatous inflammation, as well as rapid epithelialization [22].

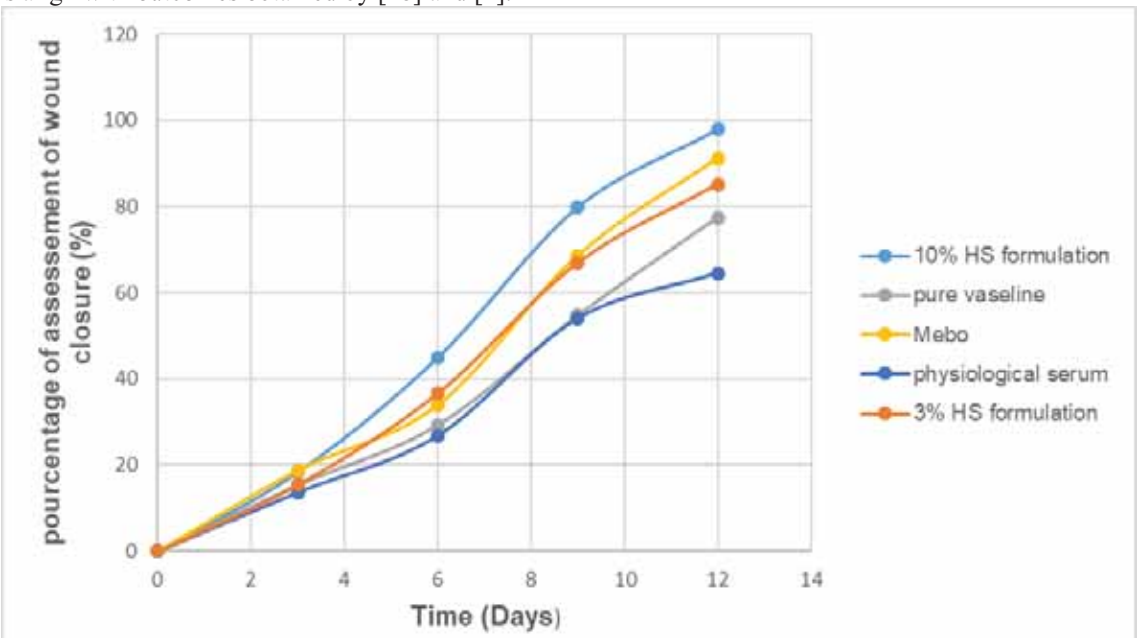



**FIGURE 3**
**Percentage of wound areas contraction of different group of rats (n=5, p-value < 0.05).**

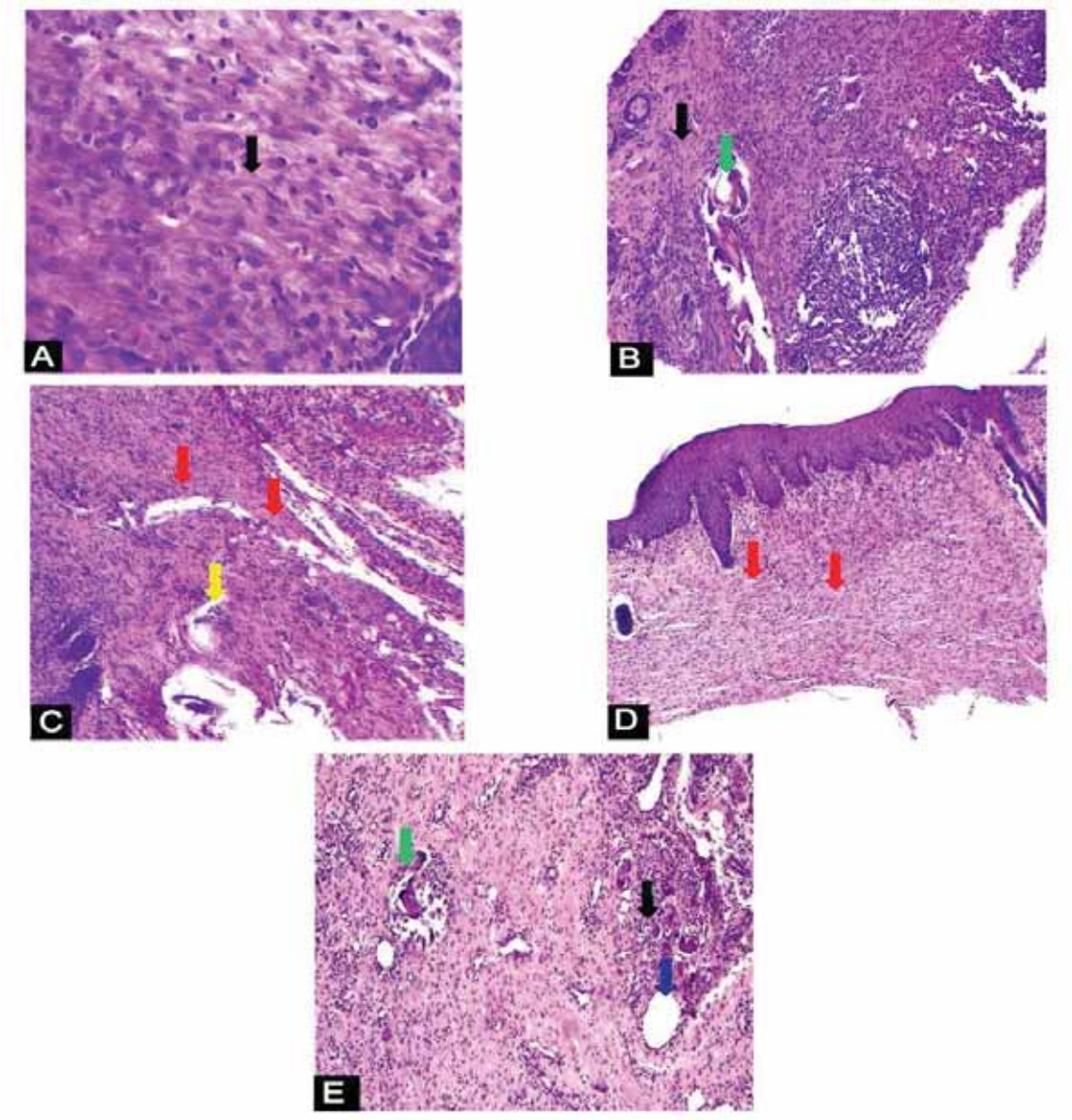



**FIGURE 4**

**Effects of the physiological serum, Mebo, 10% and 3% formulation, and control group on the histological evolution. The following is indicated by arrows.**

**(A) control group: rats treated with a physiological saline solution. (B) rats treated with pure vaseline. (C) rats treated with Mebo cream (D) rats treated with 10% Hs formulation.. (E) rats treated with 3% Hs formulation.**

**: inflammatory infiltrate.** **: Collagen formation.**

**: multinucleated giant cells.** **: Granulomatous inflammation.**

**: FBR.**

We failed to observe any hair follicles, though.

The histogram for the 10% formulation-treated group revealed a Re-epithelialization with lining thick epithelium, the existence of hair follicles, along with a broad dispersion of recently developed blood vessels with a negligible amount of inflammatory infiltrate [23], [24].

Light microscopy is used to look at the micrograph sections and evaluate the inflammatory characteristics, including tissue congestion, inflammatory cell infiltrates, and the formation of new blood vessels.[25]

The histological analysis of the epidermis revealed that both the Mebo group and the group re-

ceiving the 10% formulation (treated groups) outperformed other groups in terms of healing rates and dimension, demonstrating their combined efficacy and potential for accelerated wound healing. The dual groups displayed a well-developed epidermis with thick epithelium, along with freshly created, well-organized blood vessels and wound healing that was nearly complete [22], [26], and the presence of connective tissue associated with significant enhancements in collagen fiber arrangement and deposition as shown in Figure 4, which supports tissue regeneration by promoting and accelerating the wound healing process.[27]

H. scoparia aqueous extract contains complex chemicals such phenolic substances which possess anti-inflammatory, antioxidant, and protective properties against infectious agents. These chemicals also play a significant part in mast cell activation, which aids in epithelial contraction and, consequently, quick healing. Rapid, intensive epithelialization and capillary proliferation are produced by this formulation of an aqueous extract of H. scoparia combined with an antibacterial medication [28], [29].

## CONCLUSION

In order to establish a conducive environment that accelerates wound closure, we evaluated our formulation of H. scoparia aqueous extract in vivo on Wistars rats. According to this study, the anterior part of H. scoparia aqueous extract exhibited antioxidant activity in vitro. Application of an ointment based on its aqueous extract accelerated wound healing and re-epithelialization and significantly increased hydroxyproline rate at the wound site. The mentioned effects might be explained by their bioactive compounds and antioxidant properties. It's interesting that these characteristics imply that an ointment based on H scoparia could serve as a new medicine for treatments in wound healing applications.

## ABBREVIATIONS

**Hs:** Hammada scoparia

**FRAP:** Ferric reducing antioxidant power assay

**DPPH:** 1,1-diphenyl-2-picrylhydrazyl scavenging activity

**ANOVA:** Analysis of variance

**FBR:** foreign body reaction

## ACKNOWLEDGEMENTS

Thank everyone who contributed to this article.

The authors acknowledge the Department of Biology, Faculty of Sciences, University of Saida Dr-MoulayTahar, 20000 Saida, Algeria and Government of Algeria for funding this study

## REFERENCES

[1] Eleroui, M., Feki, A., Hamzaoui, A., Kammoun, I., Bouhamed, M., Boudawara, O. and Amara, I.B. (2021) Preparation and characterization of a novel hamada scoparia polysaccharide composite films and evaluation of their effect on cutaneous wound healing in rat. International Journal of Pharmaceutics. 608, 121056.

[2] Hou, B., Qi, M., Sun, J., Ai, M., Ma, X., Cai, W. and Qiu, L. (2020) Preparation, characterization and wound healing effect of vaccarin-chitosan nanoparticles. International Journal of Biological Macromolecules. 165, 3169-3179.

[3] Natarajan, S., Williamson, D., Stiltz, A.J. and Harding, K. (2000) Advances in wound care and healing technology. American Journal of Clinical Dermatology. 1, 269-275.

[4] Rice, J.B., Desai, U., Cummings, A.K.G., Birnbaum, H.G., Skornicki, M. and Parsons, N.B. (2014) Burden of diabetic foot ulcers for medicare and private insurers. Diabetes Care. 37(3), 651-658.

[5] Boakye, Y.D., Agyare, C., Ayande, G.P., Titiloye, N., Asiamah, E.A. and Danquah, K.O. (2018) Assessment of wound-healing properties of medicinal plants: The case of Phyllanthus muellerianus. Frontiers in Pharmacology. 9, 945.

[6] Zhang, W., Qi, X., Zhao, Y., Liu, Y., Xu, L., Song, X. and Hou, M. (2020) Study of injectable Blueberry anthocyanins-loaded hydrogel for promoting full-thickness wound healing. International Journal of Pharmaceutics. 586, 119543.

[7] Nyarko, A. K., Asiedu-Gyekye, I. J., & Sittie, A. A. (2005). A Manual of Harmonised Procedures for Assessing the Safety, Efficacy and Quality of Plant Medicines in Ghana. Sponsored by the Ghana National Drugs Programme in collaboration with Traditional and Alternate Medicine Directorate, Ministry of Health, Ghana.

[8] Haida, S. and Kribii, A. (2020) Chemical composition, phenolic content and antioxidant capacity of Haloxylon scoparium extracts. South African Journal of Botany. 131, 151-160.

[9] Bouaziz, A., Mhalla, D., Zouari, I., Jlaiel, L., Tounsi, S., Jarraya, R. and Trigui, M. (2016) Antibacterial and antioxidant activities of Hammada scoparia extracts and its major purified alkaloids. South African Journal of Botany. 105, 89-96.

[10] DjamaI, S., Otemani, A., Abbes, A., Nani, A. and Bahiani, M. (2021) Evaluation of anti-oxidant and anti-inflammatory activity of Hammada scoparia (in vitro and in vivo). Doctoral dissertation, University Ahmed Draya Faculty of Sciences and Technology, Adrar.

[11] Lamchouri, F., Benali, T., Bennani, B., Toufik, H., Hassani, L.I.M., Bouachrine, B. and Lyoussi, B. (2012) Preliminary phytochemical and antimicrobial investigations of extracts of Haloxylon scoparium. Journal of Materials and Environmental Science. 3(4), 754-759.

[12] Najjaa, H., Arfa, A.B., Máthé, Á. and Neffati, M. (2017) Aromatic and medicinal plants of Tunisian arid and desert zone used in traditional medicine, for drug discovery and biotechnological applications. Medicinal and Aromatic Plants of the World-Africa. 3, 157-230.

[13] Olugbami, J.O., Gbadegesin, M.A. and Odunola, O.A. (2015) In vitro free radical scavenging and antioxidant properties of ethanol extract of Terminalia glaucescens. Pharmacognosy Research. 7(1), 49.

[14] Gonçalves, S., Grevenstuk, T., Martins, N. and Romano, A. (2015) Antioxidant activity and verbascoside content in extracts from two uninvestigated endemic Plantago spp. Industrial Crops and Products. 65, 198-202.

[15] Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N. and Ferchichi, A. (2012) Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. Journal of Medicinal Plants Research. 6(32), 4724-4730.

[16] Lachkar, N., Lamchouri, F., Bouabid, K., Boulfia, M., Senhaji, S., Stitou, M. and Toufik, H. (2021) Mineral composition, phenolic content, and in vitro antidiabetic and antioxidant properties of aqueous and organic extracts of Haloxylon scoparium aerial parts. Evidence-Based Complementary and Alternative Medicine. 2021.

[17] Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews. 4(8), 118.

[18] Xu, N., Wang, L., Guan, J., Tang, C., He, N., Zhang, W. and Fu, S. (2018) Wound healing effects of a Curcuma zedoaria polysaccharide with platelet-rich plasma exosomes assembled on chitosan/silk hydrogel sponge in a diabetic rat model. International Journal of Biological Macromolecules. 117, 102-107.

[19] Ktari, N., Trabelsi, I., Bardaa, S., Triki, M., Bkhairia, I., Salem, R.B.S.B. and Salah, R.B. (2017) Antioxidant and hemolytic activities, and effects in rat cutaneous wound healing of a novel polysaccharide from fenugreek (Trigonella foenum-graecum) seeds. International Journal of Biological Macromolecules. 95, 625-634.

[20] Song, Y., Nagai, N., Saijo, S., Kaji, H., Nishizawa, M., & Abe, T. (2018) In situ formation of injectable chitosan-gelatin hydrogels through double crosslinking for sustained intraocular drug delivery. Materials Science and Engineering: C. 88, 1-12.

[21] Süntar, I., Tumen, I., Ustün, O., Keleş, H. and Akkol, E.K. (2012) Appraisal on the wound healing and anti-inflammatory activities of the essential oils obtained from the cones and needles of Pinus species by in vivo and in vitro experimental models. Journal of Ethno-pharmacology.139(2), 533-540.

[22] Agubata, C.O., Okereke, C., Nzekwe, I.T., Onoja, R.I. and Obitte, N.C. (2016) Development and evaluation of wound healing hydrogels based on a quinolone, hydroxypropyl methylcellulose and biodegradable microfibres. European Journal of Pharmaceutical Sciences. 89, 1-10.

[23] Alam, P., Shakeel, F., Anwer, M.K., Foudah, A.I. and Alqarni, M.H. (2018) Wound healing study of eucalyptus essential oil containing nanoemulsion in rat model. Journal of Oleo Science. 67(8), 957-968.

[24] Perini, J.A., Angeli-Gamba, T., Alessandra-Perini, J., Ferreira, L.C., Nasciutti, L.E. and Machado, D.E. (2015) Topical application of Acheflan on rat skin injury accelerates wound healing: a histopathological, immunohistochemical and biochemical study. BMC Complementary and Alternative Medicine. 15(1), 1-8.

[25] Suh, H., Lee, A.Y., Park, E.J. and Hong, J.P. (2016) Negative pressure wound therapy on closed surgical wounds with dead space: animal study using a swine model. Annals of Plastic Surgery. 76(6), 717.

[26] Toppo, F.A. and Pawar, R.S. (2016) Appraisal on the wound healing activity of different extracts obtained from Aegle marmelos and Mucuna pruriens by in vivo experimental models. Nigerian Journal of Clinical Practice. 19(6), 753-760.

[27] Wang, H., Gong, X., Guo, X., Liu, C., Fan, Y.Y., Zhang, J. and Li, W. (2019) Characterization, release, and antioxidant activity of curcumin-loaded sodium alginate/ZnO hydrogel beads. International Journal of Biological Macromolecules. 121, 1118-1125.

[28] Rosato, A., Vitali, C., De Laurentis, N., Armenise, D. and Milillo, M.A. (2007) Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. Phytomedicine. 14(11), 727-732.
[29] Estevão, L.R.M., Medeiros, J.P.D., Simões, R.S., Arantes, R.M.E., Rachid, M.A., Silva, R.M.G.D. and Evêncio-Neto, J. (2015) Mast cell concentration and skin wound contraction in rats treated with Brazilian pepper essential oil (Schinus terebinthifolius Raddi). Acta Cirurgica Brasileira. 30, 289-295.

**Received:** **22.08.2023**
**Accepted:** **09.09.2023**

## CORRESPONDING AUTHOR

**Ibtissam Gheffari**
Laboratory of Biotoxicology
University of Saida Dr- Moulay Taher
Saida 20 000 – Algeria

e-mail: ibtissam.gheffari@univ-saida.dz
igheffari@gmail.com

-© by PSP Volume 32– No. 09/2023 pages 3028-3036 Fresenius Environmental Bulletin

# *Streptomyces Lasiicapitis* KSA18 ISOLATED FROM SAHARAN SAND IN ALGERIA EFFECTIVE AGAINST MOST BACTERIA AND FUNGI

**Taha Ahmed Benabbou[1,2], Lotfi Ghellai[1,2,*], Mokhtar Benreguieg[1,2], Ayse Ozdimir[3], Keriman Gunaydin[4], Joachim Wink[5]**

[1]Departement of Biology, Faculty of Sciences, University of Saida-Dr. Moulay Tahar, 20000 Saida, Algeria
[2]Laboratory of Biotoxicology, Pharmacognosy and Biological Recovery of Plants, University of Saida-Dr. Moulay Tahar, 20000 Saida, Algeria
[3]Department of Chemistry, Istanbul Technical University, Istanbul, Turkey
[4]Department of Biology, Istanbul University, Turkey
[5]Microbial Strain Collection, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, Braunschweig D-38124, Germany

## ABSTRACT

Saharan soils continue to be a considerable source of microorganisms producing secondary metabolites and enzymes of great importance. The present study aimed to screen-out the soil samples of unexplored Saharan site of Algeria to isolate Actinobacteria species possessing antibacterial and antifungal activities. Twenty-three Actinobacteria strains were isolated from sand samples collected from Saharan fields in Algeria. The isolates were screened for antimicrobial activity. After the initial screening, 79% of the isolates showed antimicrobial activity. Strain KSA18 was selected for its potent activity. The bioactive metabolite was extracted with ethyl acetate and tested against pathogenic microorganisms by disk diffusion method. The crude extract was partially purified by column chromatography and assayed for its antimicrobial activity. Fraction A1 showed good activity against *Staphylococcus aureus* (3.25 mg/mL) and *Listeria monocytogenes* (4 mg/mL). This strain was identified as *Streptomyces lasiicapitis* by 16S rRNA sequencing. GC-MS analysis of the crude extract revealed the presence of about 17 different volatile compounds). Some of them could be directly responsible for the antibacterial or antifungal activity. The most important compounds are phenol, 2,4-bis(1,1-dimethylethyl), 3-isobutylhexahydropyrrolo[1,2-A]pyrazine-1,4-dione, 2-methyloctacosane, and dibutyl phthalate. *Streptomyces lasiicapitis* KSA 18 was effective against most of the pathogens tested. Due to its richness in various bioactive compounds, this Actinobacteria could find wide application in the biotechnology industry. However, this study requires other approaches to identify these bioactive molecules.

**KEYWORDS:**
*Streptomyces,* Saharian soil, antibacterial activity, antifungal activity, bioactive compounds

## INTRODUCTION

In 1945, Abraham and Chain discovered a substance produced by a *Colibacillus* that completely stopped the effect of penicillin, and named it penicillinase. Fleming cautioned the public in a June 26, 1945, New York Times article that overuse of penicillin could result in the development and dissemination of resistant bacteria. After five years, in Paris and London, half of the *Staphylococcus* strains were penicillin-resistant [1].

The issue of antibiotic resistance quickly spread beyond its initial outbreak, becoming a global concern. In fact, during the World Health Day event held on April 7, 2011, the WHO director-general Margaret Chan declared a warning: "...if we do not take immediate action to address this issue and safeguard our progress, we will be entering a post-antibiotic era in which many common infections will become untreatable and lethal once again." Given the gravity of the situation, the ultimate solution to combat microbial resistance is to research and develop new antimicrobial molecules. This can be achieved through the synthesis of new molecules or hemi-synthesis of known structures. Additionally, non-culturable microbial populations can be harnessed through metagenomics, which involves extracting DNA from environmental samples (the metagenome) and cloning it into vectors that are transformed into host cells. The next step involves analyzing the environmental DNA libraries for their biological activities [2]. Additionally, new bacterial or fungal species are isolated from ecosystems that have been either inadequately or not at all explored. These newly discovered species are then subjected to analysis of their fermentation products.

In their 1984 review of actinomycete isolation, Goodfellow and Haynes [3] found that only 10% of these organisms occur naturally. Nevertheless, the vast majority of antibiotics used today are derived from the natural products generated by actinomycetes and fungi [4, 5]. Actinomycetes have been

sourced from both marine sediment and soil. Despite a half-century of soil analysis by the pharmaceutical industry, only a minuscule fraction of the planet's surface has been examined.

Only a small portion of all actinomycete species have been uncovered in past research [6, 7]. Our current focus lies in studying actinomycetes, as they possess the greatest potential for generating antimicrobial metabolic products.

## MATERIALS AND METHODS

**Isolation of Actinobacteria.** Barren soil samples were collected at Kasdir (33° 42′ 35”, 1° 21′ 32”) in Naama, Algeria. Remove the top 5 cm of dirt with a sterile spatula. Then pick up 100-150 g of dirt with a sterile spatula at a depth of 5-15 cm and place on sterile aluminum paper; 50 g were shipped to the laboratory as described by Pochon and Tardieux [8] **.** Soil samples were air-dried at room temperature for one week. 9 mL of double distilled sterile water contains 1 g of dirt. Diluted $10^{-5}$. $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$ were spread on starch-casein agar. Added Nalidixic Acid and Actidion to inhibit bacterial and fungal growth. Incubate at 28-30°C for 10 days. Actinobacterial colonies were purified on ISP2 medium according to their morphological characteristics. In our pilot-scale screen, 23 actinomycetes, KSA 1-23, were isolated.

**Microbial organisms.** Gram-negative bacteria, Gram-positive bacteria and fungi used in this study were provided by the Institut Pasteur, Algiers: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC13311), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC) 19115), *Bacillus subtilis* (ATCC 10876), *Enterococcus faecalis* (ATCC 29212), Methicillin-Resistant *S. aureus* (MRSA) (ATCC 43300), *Candida albicans* (ATCC 2019) and *Aspergillus flavus* (ATCC 9643). Bacteria and fungi were grown in Mueller-Hinton and Sabouraud broths at 37°C and 25°C for 24 and 72 hours, respectively.

**Antibacterial activity.** Actinomycetes were grown on Bennett's Sand GLM agar for 7 days [9]. Using hollow plungers, 3 mm agar cylinders were placed on Mueller Hinton medium (Merck) inoculated with each test bacterium. After 4 hours at 4°C, the dishes were placed at 37°C for 24 hours before measuring the inhibition diameter [10].

**Antifungal activity.** Actinomycetes isolates were tested against filamentous fungi from the Mycological Department of the Institut Pasteur. Activity was tested using 9 g l-1 Bactocasitone, 5 g.l-1 yeast extract, 10 g.l-1 sodium citrate, 20 g l-1 glucose, 3.34 g l-1 disodium hydrogen phosphate, 0.54 g l-1 potassium di-hydrogen phosphate, and 18 g l-1 agar [11]**.** After 24-48 h at 28 °C, measure the zone of inhibition for yeast and filamentous fungi.

**DNA extraction, PCR and 16S rRNA sequencing.** DNA extraction kit was used for 0.5 mL KSA18 liquid culture (Stratec Molecular Invisorb Spin Plant Kit, Berlin, Germany). DNA extraction efficiency was tested using agarose gel electrophoresis. 16S rRNA was amplified in a thermal cycler using two primers (27F: 5′-AGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-ACGGCTACCTGTTAGGACTT-3′). DSMZ sequencing of PCR products was performed at the DSMZ center (Brauchweig, Germany). Nucleotide sequences were aligned to NCBI's GENBANK database using BLAST.

**Fermentation.** Initial screening revealed that this isolate (named KSA18) had a zone of inhibition, indicating antimicrobial activity. ISP-2 broth yields bioactive substances. 125 mL of broth was sterilized in a 250 mL Erlenmeyer flask. Sterile broth containing 4% of the two-day-old stock inoculum was stored at 28 °C and 150 rpm for 7 days. After incubation, filter the broth through Whatman N°1. Centrifuge for 15 minutes and separate the filtrate.

**Extraction and purification.** The culture filtrate (800 mL) was extracted twice with ethyl acetate and the combined extracts were evaporated to dryness under reduced pressure. Antibacterial components were purified by silica gel column chromatography (2.5×25). Use 100-200mm silica gel to pack the column. Methanol and ethyl acetate (6-4 v/v) eluted. 5 g of this crude extract was diluted in 50 mL methanol and passed through a silica gel column at 0.2 mL/min; 25 fractions (5 mL each) were collected and tested for antibacterial activity [12].

**Thin-layer chromatography combined with bioautography.** Samples were spotted on 20 cm 20 cm silica gel plates (Si60, Merck Art. 5735, Kiessel gel 60F254), washed with methanol and acetate ethyl (6:4, v/v), and air-dried overnight at 37°C. Fractionation experiments used two plates. First, bioactive chemicals were localized using retention factor (Rf) [13]. Spraying sulfuric vanillin (vanillin/H2SO4/ethanol 3:3:100, w/v/v) visualized these molecules. The second plate was placed over *S. aureus*-seeded Mueller–Hinton media (Merck) at 37°C for 24 hours. Antifungal compounds were discovered by matching their Rf on reference TLC plates to *S. aureus*-free regions.

**Identification of bioactive metabolites using GC-MS analysis.** The chemical composition of the TLC active eluent molecule was discovered by GC-MS [14]. The program ranged from 40–280 °C, and

250 °C was chosen for injection. The GC had a capillary column and a 1 l injection volume. The sample flowed at 1 mL/s and 36.5 cm/s [15]. The results were compared to NIST 11.

**Determination of minimum inhibitory concentrations.** Minimum inhibitory concentrations (MICs) of pure bioactive compounds were determined using agar dilution. Inoculation on Mueller-Hinton medium for bacteria and Sabouraud medium for yeasts and filamentous fungi with 10, 20, 30, 50, 75, and 100 g/mL of active compounds. After 24–48 hours at 37°C for bacteria and 48–72 hours at 28°C for fungi, growth plates were examined to find the lowest antibiotic concentration that inhibited the growth of each organism. Mueller-Hinton and Sabouraud medium without active ingredients and target microorganisms were used as controls.

## RESULTS

**Isolation and Preliminary screening.** Soil samples were taken at Kasdir in Naama Algeria. 1 g of soil was dried for actinomycete isolation. The 23 probable actinomycetes were isolated and purified in ISP-2. The pure colonies were maintained at 4°C on an ISP-2 layer. The isolated cultures were KSA 1 to 23. All the isolates were tested for bacteria and fungi. Antibacterial and antifungal activity was assessed by diffusion on Mueller–Hinton for bacteria and YMA and casiton for fungi. At the first screening, 34% of the strains showed weak activity, 22% showed moderate activity, 25% showed promising activity, and 19% showed no hostile activity (Table 1). 8 strains with good activity were also screened for cultural characteristics (Table 2). The antibacterial chemical KSA18 suppressed the development of bacteria and fungi. KSA18 was selected based on early screening results to investigate its extraction and antibacterial property. The methanol extract of KSA18 was screened against bacteria and fungi.

**Genetic Identification of KSA18 strain, Phylogenetic Analysis and Clustering.** Sequencing results of the 16S rRNA gene of the KSA18 strain were compared with BLAST [16]. ClustalW [17] was used to align these homologous sequences with the 16S rRNA gene sequence of KSA18. Figure 1 shows the phylogenetic tree based on 16S rRNA gene sequences using MEGA X [18], illustrating the connections between the KSA18 strain and the genus *Streptomyces*. The 16S rRNA sequence of bacterium KSA18 is 99.17% comparable to the sequence of *Streptomyces lasiicapitis* (accession number SUB11207111).

**TABLE 1**
**Primary screening of actinomycetal isolates by the agar cylinder method.**

| | Test microorganisms | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *B subtilis* | *S aureus* | *L monocytogenes* | *MRSA* | *E coli* | *P. aeruginosa* | *E faecalis* | *S. typhimurium* | *Calbicans* | *A flavus* |
| KSA 1 | – | – | – | – | – | – | – | – | – | – |
| KSA 2 | – | + | + | – | – | – | + | – | – | – |
| KSA 3 | – | – | – | – | – | – | – | – | – | – |
| KSA 4 | + | + | – | – | + | – | + | – | – | – |
| KSA 5 | – | + | – | – | + | – | + | – | – | – |
| KSA 6 | + | + | + | + | + | – | – | + | – | + |
| KSA 7 | – | – | + | – | + | + | + | – | – | – |
| KSA 8 | + | + | – | – | + | – | – | – | – | + |
| KSA 9 | – | – | – | – | – | – | – | – | – | – |
| KSA 10 | + | + | – | – | – | + | – | – | – | – |
| KSA11 | – | + | – | – | – | – | – | + | – | – |
| KSA12 | + | + | + | – | + | – | + | – | – | – |
| KSA13 | – | + | + | – | + | – | – | + | – | + |
| KSA14 | + | – | + | – | – | – | + | – | – | – |
| KSA15 | – | – | – | – | – | – | – | – | – | – |
| KSA16 | + | + | + | – | – | – | + | – | – | – |
| KSA17 | – | + | + | – | – | – | – | + | – | – |
| KSA18 | + | + | + | + | – | + | + | + | – | + |
| KSA19 | + | + | + | + | + | + | + | + | – | + |
| KSA20 | + | + | + | + | – | – | + | + | - | – |
| KSA21 | + | – | – | – | – | + | – | – | + | – |
| KSA22 | – | + | – | – | + | – | – | – | - | – |
| KSA23 | + | + | – | – | – | – | + | + | – | – |

**- no inhibition; + inhibition**

**TABLE 2**
**Colony characteristics of most active isolates cultured on Isp2.**

| Culture code | Colour | Mycelium type | Pigment production | Gram's reaction |
|---|---|---|---|---|
| KSA6 | White | Substrate | Orange | + |
| KSA7 | White | Aerial | - | + |
| KSA8 | Yellow | Aerial | Red | + |
| KSA13 | Grey | Substrate | Yellow | + |
| KSA14 | White | Aerial | Yellow | + |
| KSA16 | Green | Aerial | - | + |
| KSA18 | White | Substrate | Yellow | + |
| KSA19 | Yellow | Substrate | Yellow | + |

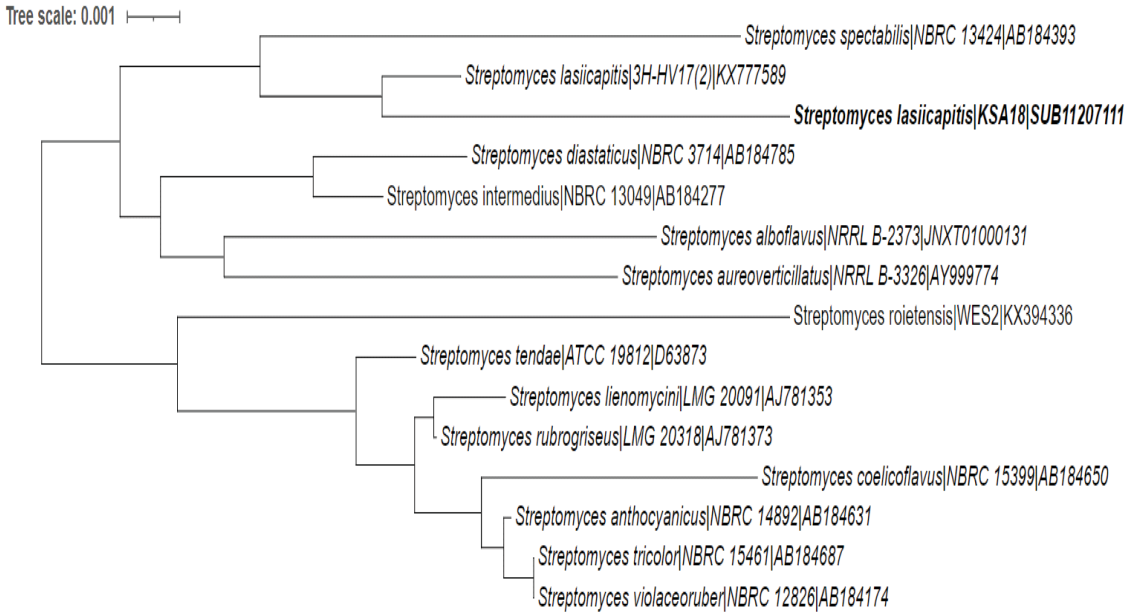



**FIGURE 1**
**A Cladogram, using neighbour-joining method of selected 16S rRNA gene sequences of the genus Streptomyses, obtained from BLAST hits, showing relationships between strains KSA18 and some closely related representative members.**

**Extraction and purification of fermentation products.** Fermentation of KSA18 lasted for 120 hours at 28 °C. The culture supernatant was collected and centrifuged. Ethyl acetate (1:2, v/v) was used for extraction, which was then evaporated to dryness to give a yellow coloured precipitate. In 50 mL methanol, 5 g of the precipitate was separated by column chromatography with methanol and ethyl acetate (6:4,v/v). 25 5-mL portions were taken.

The active portions ranged from No. 14 to No. 27. Silica gel column chromatography was used to remove unwanted impurities and metabolites.

The fractions obtained were analyzed by TLC. Ays1 (122 mg) and Ays2 (21 mg) formed the major fractions. The fraction Ays1 was the most antimicrobial.

**Growth and antimicrobial production.** The synthesis of active metabolites of KSA18 was measured for 120 hours. Antibiotic production was dependent on the growth phase, with the best efficiency of the product obtained at the end of the exponential phase and in the stationary phase. The highest yield was obtained after 72 hours of incubation with an inhibition zone of 22 mm (Figure 2).

**UV-visible analysis.** The UV spectrum of the methanol crude extract showed a pronounced absorption at 205, 210 and 240 nm. The UV absorption maximum was found at 205 nm with another peak at 240 nm, confirming a non-polyenic nature of the molecule (Figure 3).

**Minimal Inhibitory Concentration.** The purified chemical compound showed antibacterial and antifungal activity on a broad spectrum of microorganisms tested. The MIC for *Bacillus subtilis* and *Listeria monocytogenes* was 22 and 24 mg/mL, respectively. The MIC for Gram-negative bacteria was 53–62 mg/mL. The minimum inhibitory concentration of fungi was the highest compared to bacteria (71 mg/mL for *Candida albicans* and 92 mg/mL for *A. flavus*) (Table 3).

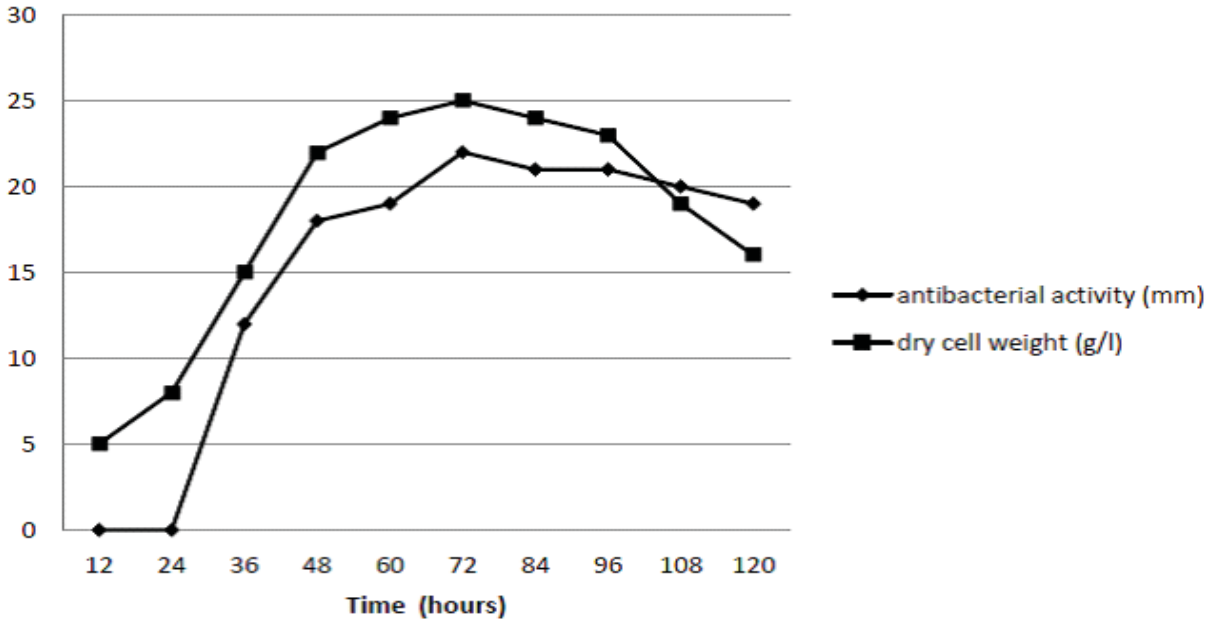



**FIGURE 2**
**Growth and antimicrobial time production KSA18 in ISP2.**

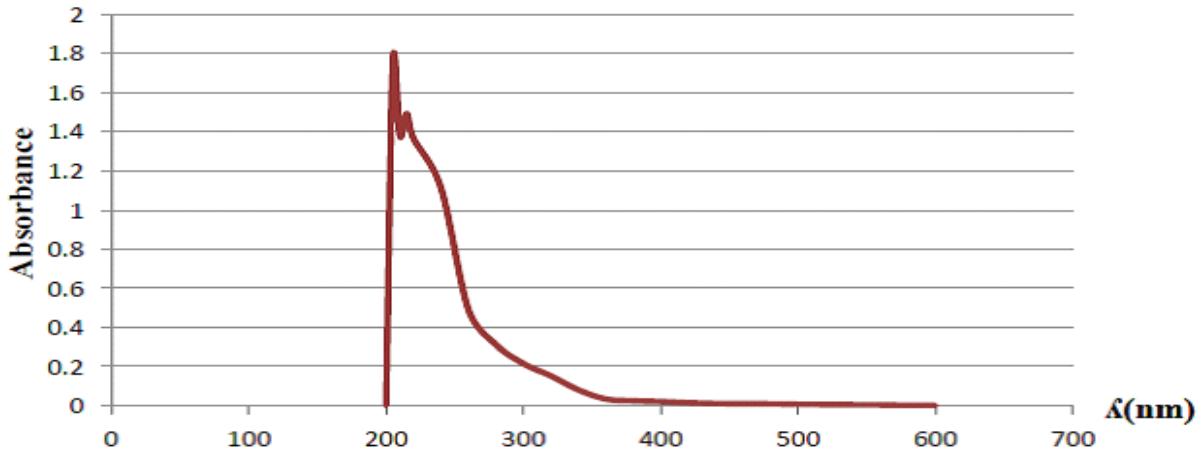



**FIGURE 3**
**The UV-Visible spectrum of the KSA18 crude extract**

**TABLE 3**
**Minimum inhibitory concentrations (MIC) of the purified bioactive metabolite isolated from *Streptomyces* KSA18.**

| Test organisms | MIC (mg/mL) |
|---|---|
| *Aspergillus flavus* | 92 |
| *Bacillus subtilis* | 22 |
| *Candida albicans* | 71 |
| *Escherichia coli* | 55 |
| *Enterococcus faecalis* | 26 |
| *Listeria monocytogenes* | 24 |
| MRSA | 37 |
| *Pseudomonas aeruginosa* | 62 |
| *Staphylococcus aureus* | 36 |
| *Salmonella Typhimurium* | 53 |

**GC-MS spectrum.** Analysis by GC-MS detected various chemicals (Figures. 4 and 5). 2,4-Bis (1,1-dimethylethyl) and O-D-glucopyranoside, ß-D-fruc are important chemicals. Other compound are listed in Table 4

## DISCUSSION

23 Actinomycetes were isolated from a poor Algerian field. Isolation of actinomycetes is difficult compared to bacteria and fungi [19]. This may be attributed to the long incubation period. Strach casein agar medium containing nalidixic acid 100 $mg.l^{-1}$ and actidione 20 $mg.l^{-1}$ inhibited contaminating bacteria and fungi.

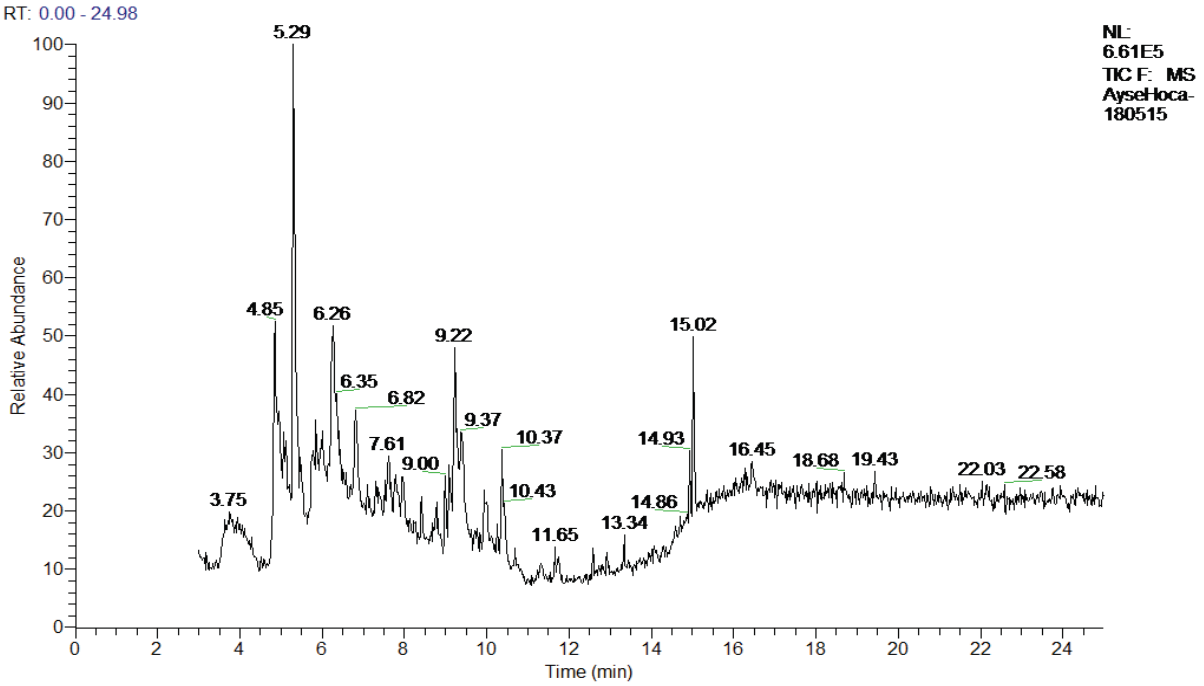



**FIGURE 4**
**GC-MS spectrum of the active fraction obtained by column chromatography of the crude extract of KSA18.**

**TABLE 4**
**GC-MS identification of bioactive compounds**

| PEAK N° | Retention Time | Identified compound | Mole. formula | Mole. weight | Activity |
|---|---|---|---|---|---|
| 1 | 4.85 | DL-Arabinose | $C_5H_{10}O_5$ | 150.05 | Antivirus activity |
| 2 | 5.29 | Xylitol | $C_5H_{12}O_5$ | 152.15 | Antimicrobial |
| 3 | 5.82 | Glucitol, 6-O-nonyl | $C_{15}H_{32}O_6$ | 308.41 | No activity reported |
| 4 | 6.26 | α- DGlucopyranoside,O-αD-gluco-pyranosyl- (1.fwdarw.3)-ß-D-fruc | $C_{18}H_{32}O_{16}$ | 504.40 | Anticarcinogenic antimutagenic |
| 5 | 6.82 | N, N-Dimethylglycine | $C_4H_9NO_2$ | 103.12 | Antioxydant |
| 6 | 7.61 | Glycerin | $C_3H_8O_3$ | | Emulsifiant |
| 7 | 9.22 | Benzofuran, 2,3-dihydro- | $C_8H_8O$ | 120.10 | anti-inflammatory activity |
| 8 | 9.29 | Maltol | $C_6H_{12}O_3$ | 126.11 | Antioxydant |
| 9 | 9.37 | Phenol, 2 , 4 Bis (1,1-Di-methylethyl) | $C_{17}H_{30}OSi$ | 278.50 | Anti-inflammatory, antioxydant, antimicrobial |
| 10 | 10.37 | Levomernthol | $C_{10}H_{20}O$ | 156.26 | Antimicrobial |
| 11 | 14.93 | 3-Isobutylhexahydropyrrolo [1,2-A] Pyrazine-1 , 4- Dione | $C_{11}H_{18}N_2O_2$ | 210.27 | Antibacterial |
| 12 | 15.02 | 2- methyloctacosane | $C_{29}H_{60}$ | 408.80 | Antimicrobial, antioxydant |
| 13 | 16.45 | Benzene propanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester | $C_{18}H_{28}O$ | 292.40 | Antioxydant |
| 14 | 18.68 | Propanoic acid | $C_3H_6O_2$ | 74.07 | antibacterial |
| 15 | 19.43 | Thieno[3,2-e] benzofuran | $C_{10}H_7NOS$ | 189.24 | Antimicrobial |
| 16 | 22.03 | Pyrrolo [1 , 2-a] pyrazine-1,4-di-one , hexahydro-3-(phenylmethyl) | $C_{14}H_{16}N_2O_2$ | 244.29 | Antioxydant |
| 17 | 22.58 | Bi s (2-ethyl hexyl) phthalat | C24H38O4 | 390.6 | Antibacterial |

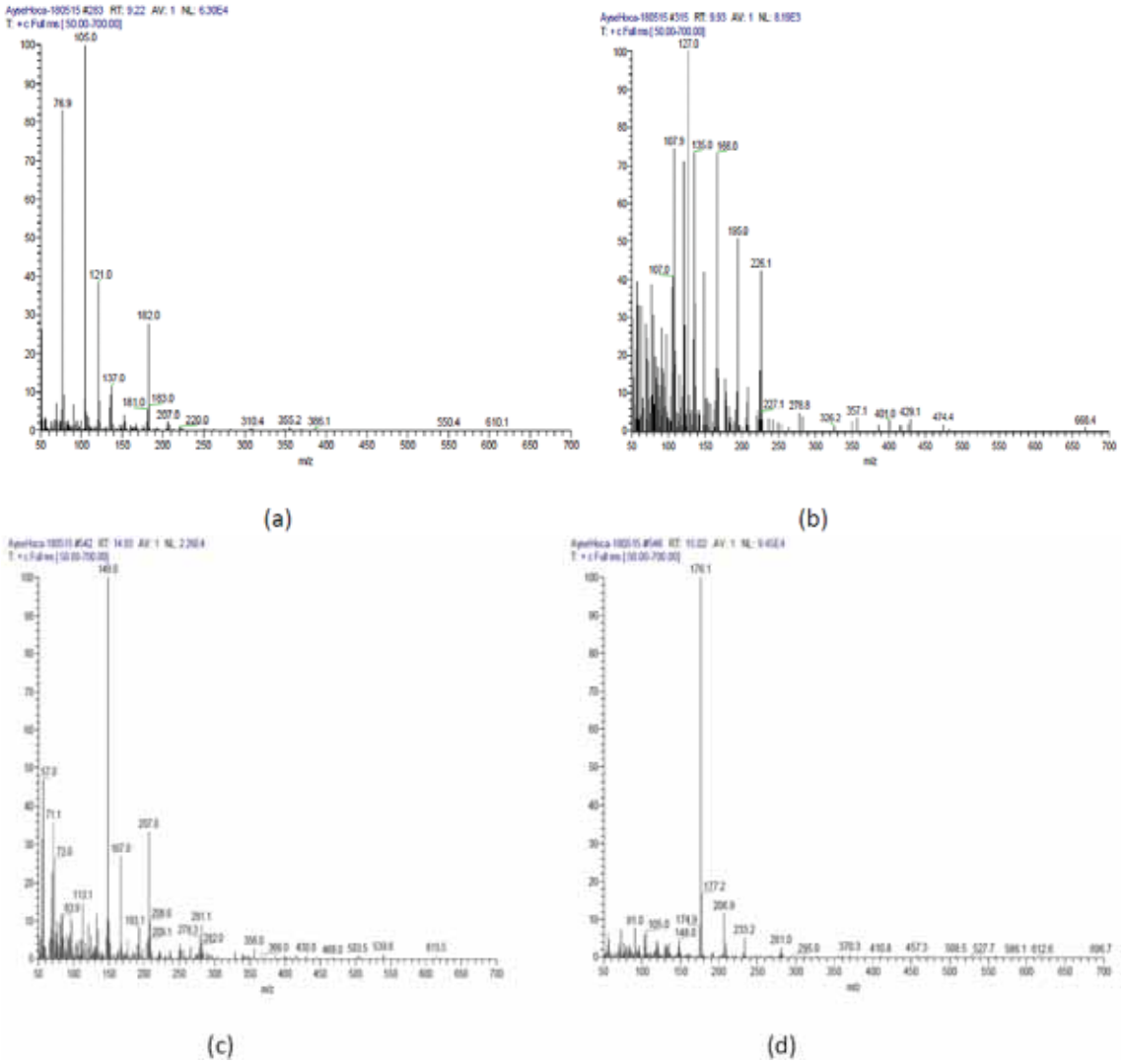



**FIGURE 5**
**GC-MS spectrum of the active fraction obtained by column chromatography of the crude extract of KSA18.**

Isolation and morphological identification showed that 79% of the identified isolates were antibacterial. In this study, only strain KSA18 was retained because it inhibited most of the bacteria studied. Morphological, physiological and biochemical characteristics were consistent with those of *Streptomyces*. The KSA18 isolate formed secondary metabolites and showed antimicrobial activity against a broad spectrum of bacteria. Valan Arasu *et al.* [20] found similar results when they isolated *Streptomyces* spp. (ERI-3) from the bedrock of the Western Ghats. The actinomycete microflora from Saharan soils in southeastern Algeria was identified using molecular methods. Then it was tested whether they kill the fungus. The extract of KSA18 *Streptomyces* showed antibacterial and antifungal activity.

The production of secondary metabolites started at the end of the exponential phase and continued during the stationary phase. We found that the production of antimicrobial compounds was significantly related to the development of the KSA18 strain. The same effect has already been demonstrated for the antimicrobial BTSS-301 derived from marine *Streptomyces coeruleorubidus* [21].

When the UV-VIS spectrum of the crude extract was checked against the maximum molecular absorbance of polyenes [22], it became clear that the absorbance peaks of the KSA18 extract did not have a polyenic structure. Since polyenes are known to be toxic and poorly soluble, this is poor for screening new antifungal compounds [23].

The TLC results showed that the best composition of the solvent used for the crude extract is ethyl acetate/methanol. Boughachiche [24] found that ethyl acetate methanol causes rapid migration of active ingredients. Therefore, it provides a good partitioning and suitable purification for the crude extract obtained with methanol.

The GC-MS analysis showed a great diversity with about 17 different volatile compounds in the active fraction of the methanolic extract. Most of them could be responsible for the biological activity. Devi

et al. showed the antifungal activity of phenol, 2,4-bis(1,1-dimethylethyl), which is produced by Actinomycete and is considered to promote the growth of mangroves [25]. 3-Isobutylhexahydropyrrolo[1,2-A] pyrazine-1,4-dione has antimicrobial activity [26]. 2-Methyloctacosan also showed antimicrobial and antioxidant activity in the work of Pelo *et al.* [27] . *Streptomyces albidoflavus* produces dibutyl phthalate, which has antimicrobial activity [28-30]. However, for this study, other methods must be used to determine the structures of the various molecules of technological and biological interest contained in our extract. The different MICs determined for our extract show its activity against most pathogenic microorganisms, revealing the presence of several interesting molecules

## CONCLUSION

Standard therapies for infections caused by multidrug-resistant strains are no longer sufficient, and even second- and third-line drugs are not sufficiently effective. Therefore, it is crucial to find new bioactive compounds of natural origin to continuously advance the discovery and development of new drugs. For this reason, the main objective of this work is to isolate actinobacterial strains and demonstrate their antimicrobial activity. A total of 23 actinomycetes were isolated from Saharan soil. Most of them showed microbial activity. Strain KSA 18 was selected for its efficacy against the majority of pathogens tested. Molecular identification of the strain allows it to be assigned to the species *Streptomyces lasiicapitis*. Submerged fermentation of the selected bacteria allows the production and extraction of metabolites. The GC-MS technique showed a richness of the extract in different bioactive compounds. This work should be continued to identify the different chemical structures of the active molecules.

## ACKNOWLEDGEMENT

The authors are grateful to the Departments of Molecular Biology and Genetics, Istanbul University, Turkey, for all their contributions to the realization of this work.

**Author Contributions.** Material preparation, data collection and analysis were performed by MB, DEA and TAB. and MB. KZ and M.B. writing original draft; AO, KG. supervision, review and editing; JW. co-supervised the work; M.B., KZ. conceptualized, revised and corrected the paper. All authors listed have made a substantial, direct and intellectual contribu-tion to the work and approved the work for publication. All authors have read and agreed to the published version of the manuscript.

**Funding.** This research received no external funding.

**Data Availability.** The datasets generated and/or analysed during the current study are not publicly available for ethical reasons, as well as privacy reasons, but are available from the author on reasonable request.

**Code Availability.** Not applicable.

## REFERENCES

[1] Briand, Y.M. (2009) A history of antibiotic resistance in six bacteria. Edition L'harmattan. Paris. Pages.
[2] Zhang, L., Demain, A.L. (2005) Integrated approaches for discovering novel drugs from natural microbial products. Natural product, drug discovery and therapeutic medicine. Totowa, New Jersey. 4, 375-382
[3] Goodfellow, M., Haynes, J.A. (1984) Actinomycetes in marine sediments. Biological, Biochemical and Biomedical Aspects of Actinomycetes. 453-472.
[4] Butler, M.S., Buss, A.D. (2006) Natural products: The future scaffolds for novel antibiotics. Biochem. Pharmacol. 71, 919-929.
[5] Newman, D.J., Cragg, G.M. (2007) Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70, 461-477.
[6] Baltz, R.H. (2005) Antibiotic discovery from actinomycetes: Will a renaissance follow the decline and fall? SIM News, 55, 186-196.
[7] Baltz, R.H. (2007) Antimicrobials from actinomycetes: Back to the future. Microbe. 2, 125-131.
[8] Pochon, J., Tardieux, P. (1962) Analytical techniques in soil microbiology. Saint Mandé: Edition de la tourtourelle. Volume(Issue), Pages.
[9] Lee, J.Y., Hwang, B.K. (2002) Diversity of antifungal actinomycetes in various vegetative soils of Korea. Can. J. Microbiol. 48, 407-417.
[10] Lemriss, S., Laurent, F., Couple, A., Casoli, E., Lancelin, J.M., Saintpierre, B.D. (2003) screening of nonpolymeric antifungal metabolites produced by clinical isolates of actinomycetes. Can. J. Microbiol. 49, 669-674.
[11] Kitouni, M., Boudemagh, A., Oulmi, L., Reghioua, S., Boughachiche, F., Zerizer, H., Hamdiken, H., Couble, A., Mouniee, D., Boulahrouf, A., Boiron, P. (2005) Isolation of actinomycetes producing bioactive substances from water, soil and tree bark samples of the north–east of Algeria. Journal de Mycologie Médicale. 15, 45–51.

[12] Aouiche, A., Sabaou, N., Meklat, A., Zitouni, A., Mathieu, F., Lebrihi, A. (2012) Activité antimicrobienne de Streptomyces sp. PAL111 d'origine saharienne contre divers microorganismes cliniques et toxino-gènes résistants aux antibiotiques. J. Mycol. Med. 22, 42-51.
[13] Mendham, J., Denney, R.C., Barnes, J.D., Thomas, M. (2006) Vogel's quantitative chemical analysis, De Boeck Ed, Brussels. Pages.
[14] Parthasarathi, S., Sathya, S., Bupesh, G., Samy, R.D., Mohan, M.R., Kumar, G.S. (2012) Isolation and characterization of antimicrobial compound from marine Streptomyces hygroscopicus BDUS 49. World J. Fish Mar. Sci. 4(3), 268-77.
[15] Barton, H.A. (2006) Introduction to cave microbiology: a review for the non-specialist. J. Caves. Karst. Stud. 68(2), 43–54.
[16] Zoetendal, E.G., Rajilić, S.M., de Vos, W.M. (2008) High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut. 57(11), 1605-1615.
[17] Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 22(22), 4673-4680.
[18] Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution. 35(6), 1547.
[19] Cavalla, M., Eberlin, T. (1994) Isolation of soil streptomycetes. The Operon. 19, 13–17.
[20] Valan, A.M., Duraipandiyan, V., Agastian, P., Ignacimuthu, S. (2009) In vitro antimicrobial activity of Streptomyces spp. ERI-3 is isolated from Western ghats rock soil (India). J. Mycol. Med. 19, 22-28.
[21] Kandula, S.K., Terli, R. (2013) Production, purification and characterization of an antimicrobial compound from marine Streptomyces coeruleorubidus BTSS-301. J. Pharm. Res 7, 397-403.
[22] Rabek, J.F. (1995) Polymer photodegradation: mechanisms and experimental methods, 1st Ed., Chapman & Hall, London. Pages.
[23] Badji, B., Riba, A., Mathieu, F., Lebrihi, A., Sabaou, N. (2005) Antifungal activity of a Saharan Actinomadura strain against various pathogenic and toxinogenic fungi. J. Mycol. Med. 15, 211-219.
[24] Boughachiche, F. (2012) Study of antibiotic molecules secreted by strains belonging to the genus Streptomyces, isolated from Sebkha," Ph.D. thesis, University Mentouri- Constantine. Faculty of Natural and Life Sciences, Algeria. Pages.
[25] Devi, T.S., Vijay, K., Vidyavathi, R.M., Kumar, P., Govarthanan, M., Kavitha, T. (2021) Antifungal Activity and Molecular Docking of Phenol, 2, 4-Bis (1, 1-Dimethylethyl)-Produced By Plant Growth-Promoting Mangrove Actinobacteria; Kutzneria Sp. Strain TSII. Pages.
[26] Sun, S., Tian, L., Wang, Y., Wu, H., Lu, X., Pei, Y. (2009) A novel natural product from the fermentation liquid of marine fungus Trichoderma atroviride G20-12. Asian J. Trad. Med. 4, 123-127.
[27] Pelo, S.P., Oluwafemi, A.A., Ezekiel, G. (2021) Chemotaxonomic profiling of fungal endophytes of Solanum mauritianum (alien weed) using gas chromatography high resolution time-of-flight mass spectrometry (GC-HRTOF-MS). Metabolomics. 17(5), 1-13.
[28] Aboobaker, Z., Viljoen, A., Chen, W., Crous, P., Maharaj, V., Van Vuuren, S. (2019) Endophytic fungi isolated from Pelargonium sidoides DC: antimicrobial interaction and isolation of a bioactive compound. S. Afr. J. Bot. 122, 535–42.
[29] Lee, D.S. (2000) Dibutyl phthalate, an -glucosidase inhibitor from Streptomyces melanosporofaciens. J. Biosci. Bioeng. 89(3), 271–3.
[30] Mangamuri, U., Muvva, V., Poda, S., Naragani, K., Munaganti, R.K., Chitturi, B. (2016) Bioactive metabolites produced by Streptomyces cheonanensis VUK-A from Coringa mangrove sediments: isolation, structure elucidation and bioactivity. 3 Biotech. 6(1), 63.

**Received:** **16.03.2023**
**Accepted:** **06.09.2023**

**CORRESPONDING AUTHOR**

**Lotfi Ghellai**
Departement of Biology,
University of Saida-Dr. Moulay Tahar
Saida 20000 – Algeria

e-mail: lotfi.ghellai@hotmail.ch
lotfi.ghelai@univ-saida.dz

- © by PSP Volume 32– No. 09/2023 pages 3037-3049 Fresenius Environmental Bulletin

# ANTIBIOTIC SUSCEPTIBILITY PATTERNS AND SEARCH FOR ESBL-PRODUCING *Pseudomonas aeruginosa* ISOLATED FROM THE FEED WATER TANK OF A DAIRY UNIT LOCATED IN BECHAR PROVINCE (ALGERIA)

**Elhassan Benyagoub**[1,2,*]

[1]Architecture and Environmental Heritage Laboratory (Archipel), Mohammed Tahri University of Bechar (08000), Bechar (Algeria)
[2]Faculty of Life and Natural Sciences, Department of Biology, Mohammed Tahri University of Bechar (08000), Bechar (Algeria)

## ABSTRACT

As an opportunistic pathogen that lives in wetlands such as water, the presence of *Pseudomonas spp* in drinking water or that intended for the agri-food industry can pose a risk to consumers. The study aims to search for *Pseudomonas spp* in the feed water tank of a dairy unit located in Bechar, and then to characterize the virulence factors of the isolates, namely antibiotics resistance, hemolytic power, and biofilm formation.

A total of 10 water samples were analyzed to isolate *Pseudomonas* by the membrane filter technique. The identification of *Pseudomonas* spp isolates was carried out using standard microbiological methods. The Kirby-Bauer diffusion test-based assessment of antibiotic susceptibility was performed.

The bacteriological analysis showed that 7/10 (*i.e.* 70%) of the water samples were contaminated with *Pseudomonas* spp. However, bacterial identification confirmed that the isolated strains belonged to the *P. aeruginosa* species. These strains showed a resistance rate of 100% to cefazolin, ticarcillin-clavulanic acid, ceftazidime, cefotaxime, cephalothin, and cefoxitin, and at a lower rate of resistance, *i.e*. 86 and 57% for amikacin and tobramycin, respectively, and sensitive to ciprofloxacin, ofloxacin, piperacillin, piperacillin-tazobactam, and gentamicin, which seemed to be the most active molecules. Based on antibiotic resistance results against the aminoglycoside, cephalosporin, and β-lactam classes, all the *P. aeruginosa* isolates were classified as multidrug-resistant (MDR). Besides, all the *P. aeruginosa* isolates show β-hemolysis and can form biofilms.

*P. aeruginosa* isolates showed a worrying degree of virulence that requires special attention from the dairy unit managers, and appropriate treatment should be conducted to eliminate any risk that could harm consumers.

**KEYWORDS:**
Bechar, Dairy unit, Feed water tank, *Pseudomonas aeruginosa*, Virulence factors

## INTRODUCTION

As a vital element for human life, essential for many human activities, and for its multiple uses in various fields as a raw and basic material in the pharmaceutical and food industries, water must therefore meet the sanitary conditions for its consumption without harming consumer health [1].

The presence of micro-organisms in the drinking water is mainly due to the deterioration of the water quality at the water point, insufficient protection or maintenance of receiving facilities, failure of the disinfection treatment, or contamination of water during transportation or storage in the network [2], microbial contamination of drinking water can transmit diseases such as cholera, dysentery, typhoid fever, poliomyelitis, and diarrheal diseases that lead to approximately 485000 consecutive deaths each year [3]. *Pseudomonas aeruginosa* strains are found in various environmental habitats and require a minimum supply of nutrients (low nutrient or oligotrophic environments) for their reproduction and survival, and also can be found in high-nutrient environments such as in sewage, which makes water a favorable environment for their development [4-6]. This bacterium is an opportunistic pathogen that can cause serious infections for immunocompromised patients, and those with chronic diseases, and can be considered a foodborne pathogen involved in food poisoning in various food groups such as water, milk, meat, fruits, and vegetables [7, 8].

The virulence and prevalence of multi-drug-resistant *Pseudomonas aeruginosa* are still of great concern for the world's public health. Single, multiple resistance, or even pandrug-resistant *Pseudomonas aeruginosa* (PDRPA), which is resistant to all commercially available antipseudomonal antimicrobial agents, is rapidly increasing [9]. Due to many factors, three of the four main classes of antibiotics have become less effective or even ineffective against *P. aeruginosa* (penicillins/cephalosporins/monobactams/carbapenems, aminoglycosides, fluoroquinolones [10, 11].

On this basis, the study aimed to isolate *Pseudomonas* spp from the feed water tank of a dairy unit

located in Bechar, and to study the antibiotic susceptibility pattern of *Pseudomonas* spp isolates, their virulence factors namely hemolytic potency, pigments production, and the ability to form biofilms.

## MATERIALS AND METHODS

All experiments were carried out at Mohammed Tahri University of Bechar (Algeria), for five months from February to June 2023.

**Studied dairy unit.** The dairy unit is located in Bechar province (Southwest of Algeria) and produces pasteurized partially skimmed milk, fermented milk, and lemonade. For water requirements, the dairy unit has a water tank built of concrete with a storage capacity of 60 $m^3$ and is fed from a 70m deep borehole [12, 13].

**Water sampling.** A total of 10 water samples from the feed water tank were analyzed to isolate *Pseudomonas* spp. The water samples were collected in sterile glass bottles following the standard sampling methods described by Rodier [14] from February to May 2023 (Table 1, Figure 1).

**TABLE 1**
**Frequency and date of water sampling from the feed water tank**

| Samples | Dates of collection |
|---|---|
| S1 (E1) | February 26, 2023 |
| S2 (E2) | March 8, 2023 |
| S3 (E3) | March 15, 2023 |
| S4 | March 22, 2023 |
| S5 | April 8, 2023 |
| S6 | April 16, 2023 |
| S7 (E9) | April 24, 2023 |
| S8 (E12) | April 30, 2023 |
| S9 (E17) | May 10, 2023 |
| S10 (E19) | May 20, 2023 |

**S (1 to 10):** Water tank samples

**FIGURE 1**
**Water tank samples (Original, 2023)**

**Detection of *Pseudomonas spp.*** Search for *Pseudomonas spp* was carried out by filtering 250 mL of water through 0,45µm-pore-size membranes (Membrane filter technique). The membranes were then placed on cetrimide-nalidixic acid agar, and the dishes were incubated at 42°C for 24 hours [15] (Figure 2).

**Identification tests for *Pseudomonas* spp.** The identification of the isolates was performed according to the standard microbiological methods given in Bergey's manual [11, 16]. Firstly, morphological characteristics on culture media, bacterial growth on nutrient agar from 37 to 42°C, observing bacterial cells under the microscope (wet mount and Gram staining). Secondly, classical bacteriological biochemical tests on TSI Agar, meat-liver glucose 0,6% agar, mannitol-motility test medium, indole test, citrate utilization test, and oxidative-fermentative test on Hugh Leifson medium (MEVAG). Other tests such as catalase test, nitrate reduction test, cytochrome C Oxidase assay, and API 20E multitest system were also applied.

**FIGURE 2**
**Membrane filter technique for isolation of *Pseudomonas* spp (Original, 2023)**

**Virulence factors of *Pseudomonas* spp.**

**Hemolysis test.** This test is not only a bacterial pathogenicity or virulence characteristic to look for, but also a tool for identifying specific microbial species.

After 24 hours of incubation at 37°C of the Columbia agar with 5% sheep blood plates incubated with the tested isolate, the hemolytic power is revealed by the presence of a characteristic clear halo at the periphery of each bacterial culture [17].

**DNase production test.** The DNase test was carried out on DNase agar. DNase agar medium incubated with the tested isolate at 37°C for 24 hours was flooded with 1,0 N HCl to precipitate. The generation of clear zones around the inoculation line was considered DNase-positive. However, lack of clearance around bacterial colonies was considered DNase-negative [18, 19].

**Pigments production.** King A agar and King B agar slants were incubated with the tested isolates at 37°C for 24 hours.

Two pigmentations are sought, namely:

-Pyocyanin (PYO): Bluish-green or blue color formation on king A agar slants medium was considered as pyocyanin pigment, which was soluble in chloroform.

-Pyoverdine (PVD): After incubation, King B agar slants incubated with the tested isolate are placed under the UV lamp (280 nm) for fluorescence detection. Yellowish green pigment formation was considered as pyoverdine pigment [20,21].

**Biofilm formation.** The tube method (TM) was conducted in this study as described by Christensen et al. [22]. It is a qualitative test for the detection of biofilm-producing microorganisms, due to the appearance of a visible film inside a polystyrene test tube containing TSB medium incubated with the tested isolate [23].

**Antibacterial susceptibility patterns and ESBL screening. Antibiotic susceptibility testing (AST).** The antibiotic susceptibility pattern of *Pseudomonas* spp isolates was evaluated using the Kirby-Bauer disk diffusion method in which the inhibition zone diameters were measured in mm and then classified as sensitive (S), intermediate (I), or resistant (R) according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI) [24].

In this study, the antibiotic discs used for the antibacterial susceptibility testing were as follows: Cefoxitin (CX 30µg), Cefotaxime (CTX 30µg), Ceftazidime (CAZ 30µg), Amikacin (AK 30µg), Gentamicin (CN 10µg), Ciprofloxacin (CIP 5µg), Aztreonam (ATM 30µg), Cefazolin (CZ 30µg), Cephalothin (KF 30µg), Ofloxacin (OF 5µg), Ticarcillin-clavulanic acid (TTC 85µg), Piperacillin-tazobactam (PIT 100/10µg), Piperacillin (PRL 100µg), and Tobramycin (TOB 10µg).

Isolates that were observed to be resistant to at least three different antibiotics were classified as multi-antibiotic-resistant isolates (MAR) [25, 26].

**Determination of the MAR index**

MAR index was calculated using the formula below [27, 28] :

$$\text{MAR index} = \frac{a}{b}$$

**Where:**

(a): Number of antibiotics to which the tested isolate is resistant

(b): Total number of antibiotics tested

**Phenotypic detection of ESBL.** According to the recommendations given by the antibiogram committee of the French Microbiology Society (CA-SFM), the ESBL test was intended for isolates that show a low level of bacterial susceptibility to a series of cephalosporins. *Escherichia coli* ATCC 25922 was used as a negative control while *Klebsiella pneumoniae* ATCC 700603 was used as a positive control strain [28].

**Synergy test.** The search for ESBL-producing *Pseudomonas aeruginosa* was performed by the disk-diffusion method for synergistic detection on Mueller-Hinton agar between an antibiotic disc containing a β-lactamase inhibitor, placed at 20 or 30mm from the central disc of a 3rd generation cephalosporins disc (cefotaxime, ceftazidime or cefepime) and aztreonam. The incubation was carried out at 37°C for 24 hours [29].

**Double-disc synergy test (DDST).** This test aims to look for an increase in the inhibition zone of a 3rd generation cephalosporins disc, preceded by the application of a disc containing amoxicillin-clavulanic acid, compared to another disc carrying the same cephalosporin and placed side by side on the Mueller-Hinton agar incubated with the tested isolate.

The Petri dishes were left for one hour at laboratory ambient temperature, and the amoxicillin-clavulanic acid disc was then replaced by a cefotaxime disc.

After incubation of Petri dishes at 37°C for 24 hours, a positive test is expressed in a 5mm increase in the zone of inhibition of the cefotaxime disc in the presence of clavulanic acid compared to the zone of inhibition of the cefotaxime disc alone [29].

**Phenotypic tests on a cloxacillin-containing agar.** It was performed on Mueller-Hinton agar containing a concentration of 250 mg/L of cloxacillin (MH-CLOXA), incubated with the tested isolate at 37°C for 24 hours [29].

•ESBL-positive bacteria: A synergy is shown between ceftazidime or cefepime and amoxicillin-clavulanic acid discs.

•ESBL-negative bacteria: The diameter of the inhibition zone around cefepime and ceftazidime discs is increased and there is no visible synergy.

**Statistical analysis.** The different ratios related to antimicrobial susceptibility testing and ESBL tests were calculated using Microsoft Excel software.

**TABLE 2**
**Search results for *Pseudomonas spp* isolates from the feed water tank**

| Samples | Bacterial isolation results |
|---|---|
| S1 (E1) | +ve (*Pseudomonas sp*) |
| S2 (E2) | +ve (*Pseudomonas sp*) |
| S3 (E3) | +ve (*Pseudomonas sp*) |
| S4 | -ve |
| S5 | -ve |
| S6 | -ve |
| S7 (E9) | +ve (*Pseudomonas sp*) |
| S8 (E12) | +ve (*Pseudomonas sp*) |
| S9 (E17) | +ve (*Pseudomonas sp*) |
| S10 (E19) | +ve (*Pseudomonas sp*) |

## RESULTS

**Isolation of *Pseudomonas* spp.** The search results for *Pseudomonas* spp from the water tank are shown in Table 2 below.

The search results for *Pseudomonas* spp showed that 7/10 (*i.e.* 70%) of the water samples taken from the feed water tank were contaminated with *Pseudomonas* spp.

**Identification of *Pseudomonas* spp isolates.** The morphological and microscopic examination results as well as the metabolic and biochemical characteristics of *Pseudomonas spp* strains isolated from the feed water tank are shown in Tables 3, 4, and Figure 3 below.

Macroscopic examination showed that the colonies of *P. aeruginosa* had a circular form shape, a regular shape, and raised relief (elevation), with a smooth texture. They are Gram-negative bacilli, motile bacteria that produce pigmentations (pyoverdine and pyocyanin).

According to Table 4, the isolated *Pseudomonas* spp strains were characterized by a strict aerobic respiratory type (oxidative metabolism OF/O), unable to ferment lactose and glucose under anaerobiosis conditions, negative for indole and DNAase tests, positive for oxidase, catalase, and esculin tests. Besides, these strains were able to use sodium citrate as the sole carbon and energy source and also showed β-hemolysis.

**TABLE 3**
**Morphological and microscopic characteristics of *Pseudomonas* spp isolates**

| | Macroscopic characteristics | | | | Microscopic characteristics | | | Pigments | | Isolation on selective culture medium |
|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial strains | Form | Shape | Elevation | Texture | Grouping mode | Motility | Gram | PVD (Under UV Light) King B agar | PYO (soluble in chloroform) King A agar | Cetrimid-nalidixic acid agar |
| *Pseudomonas* spp strains | Round | Regular | Raised | Smooth | Diplobacilli | + | Gram-negative bacilli | + | + | + |

**PVD:** Pyoverdine, **PYO:** Pyocyanin.

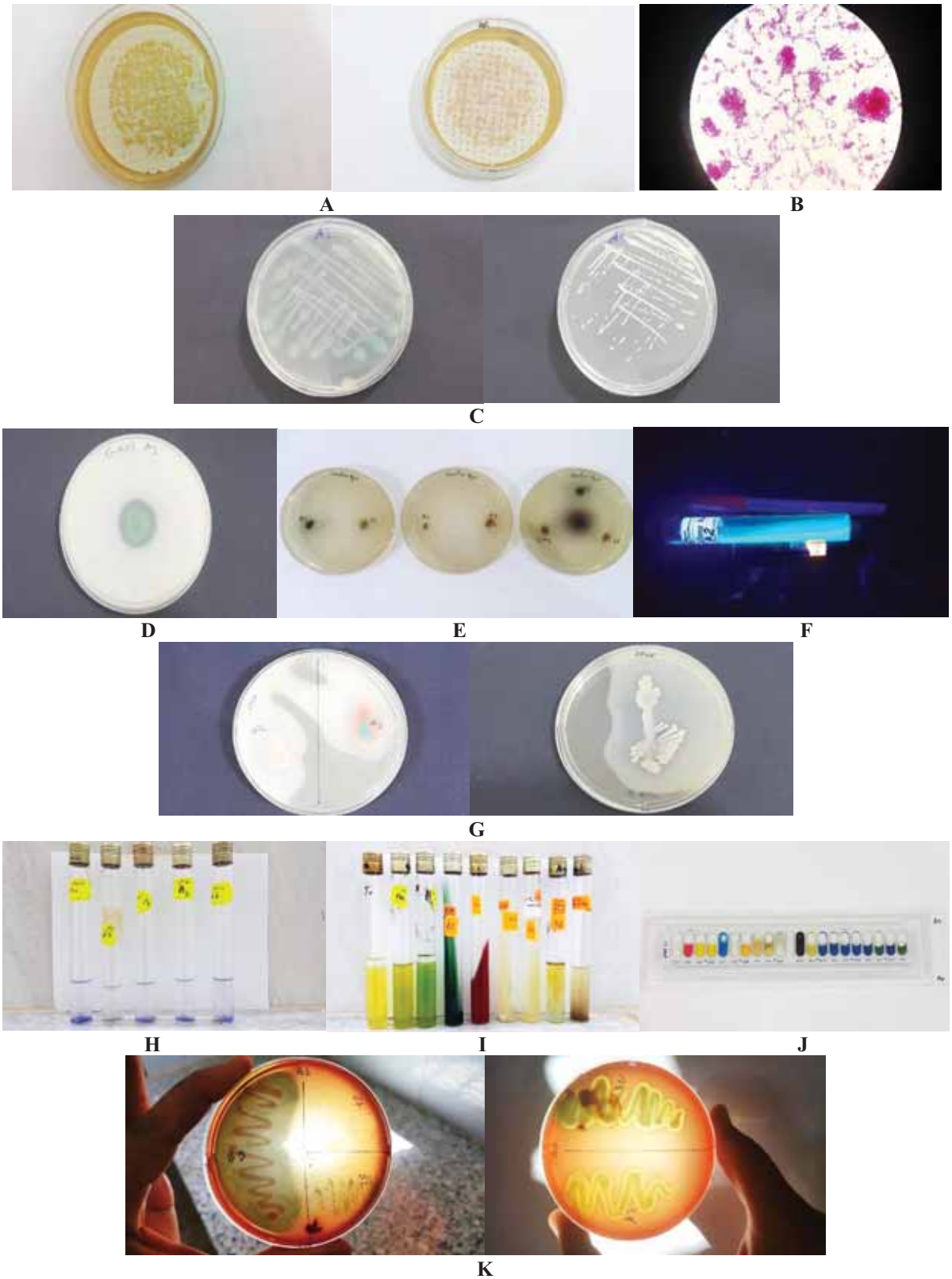


**FIGURE 3**

**Isolation and identification tests for *Pseudomonas* spp isolates (Original, 2023)**

**A:** *Pseudomonas* sp on Cetrimide-nalidixic acid agar (Membrane filter technique); **B:** Gram-negative bacilli (Gram stain); **C:** Pure culture of *Pseudomonas* sp isolate on Nutrient agar; **D:** Casein hydrolysis; **E:** Esculin hydrolysis; **F:** Pyoverdine pigment under UV light; **G:** DNase test; **H:** Oxidase test; **I:** Biochemical tests for bacterial identification; **J:** API 20 multitest; **K:** Hemolysis test. **Source: Own study.**

**TABLEAU 4**
**Biochemical and metabolic characteristics of *Pseudomonas* spp isolates**

| Bacterial strains isolated | TSI agar | | | Simmons citrate agar | Mannitol motility medium | | Indole test | Esculin hydrolysis | Nitrate reduction test | MEVAG test | | DNase test | Casein hydrolysis | Catalase test | Oxidase test | Type of cellular respiration on Meat-Liver glucose 0,6% agar medium | Hemolysis test | Gelatin hydrolysis | Casein hydrolysis | Growth on nutrient agar at 42°C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lactose | Glucose | $H_2S$ | | Mannitol | Motility | | | | OF/O | OF/F | | | | | | | | | |
| **E1** | - | - | - | + | - | + | - | + | - | + | - | - | + | + | + | OA | β-hemo | + | + | + |
| **E2** | - | - | - | + | - | + | - | + | - | + | - | - | + | + | + | OA | β-hemo | + | + | + |
| **E3** | - | - | - | + | - | + | - | + | - | + | - | - | + | + | + | OA | β-hemo | + | + | + |
| **E9** | - | - | - | + | - | + | - | + | - | + | - | - | + | + | + | OA | β-hemo | + | + | + |
| **E12** | - | - | - | + | - | + | - | + | - | + | - | - | + | + | + | OA | β-hemo | + | + | + |
| **E17** | - | - | - | + | - | + | - | + | - | + | - | - | + | + | + | OA | β-hemo | + | + | + |
| **E19** | - | - | - | + | - | + | - | + | - | + | - | - | + | + | + | OA | β-hemo | + | + | + |

**E:** Bacterial strains; **TSI agar:** Triple Sugar Iron agar; **OF:** Oxidative-Fermentative test on Hugh Leifson medium (MEVAG) (**OF/O:** Oxidative metabolism; **OF/F:** Fermentative metabolism); **OA:** Obligate aerobe; **β-hemo:** β-hemolysis; **(+):** Positive test; **(-):** Negative test.

**TABLE 5**
**Identification of *Pseudomonas* spp isolates with the API 20E multitest system**

| API test system | Samples | Bacterial species | Selective culture medium |
|---|---|---|---|
| API 20E test | S1 (E1) | *P. aeruginosa* | Cetrimide-nalidixic acid agar |
| | S2 (E2) | *P. aeruginosa* | |
| | S3 (E3) | *P. aeruginosa* | |
| | S7 (E9) | *P. aeruginosa* | |
| | S8 (E12) | *P. aeruginosa* | |
| | S9 (E17) | *P. aeruginosa* | |
| | S10 (E19) | *P. aeruginosa* | |

***P. aeruginosa*:** *Pseudomonas aeruginosa*; **S:** Samples; **E:** Bacterial strains

**Bacterial identification with the API 20 E multitest system.** The API 20E multitest results are given in Table 5 below.

The biochemical identification results using the API 20E test system confirmed that the isolates belong to the *P. aeruginosa* species with a probability ranging from 0,984 to 1.

**AST.** The antibiotic susceptibility testing results for *P. aeruginosa* strains are shown in Tables 6, 7, and Figure 4 below.

The obtained results showed a resistance rate of 100% to cefazolin, ticarcillin-clavulanic acid, ceftazidime, cefotaxime, cephalothin, and cefoxitin, and at a lower rate of resistance, *i.e*. 86 and 57% for amikacin and tobramycin, respectively, and sensitive to ciprofloxacin, ofloxacin, piperacillin, piperacillin-tazobactam, and gentamicin.

Table 7 above showed six (6) different antibiotic resistance patterns for *P. aeruginosa* which include a bacterial resistance against 6 to 10 of the 14 antibiotics tested, and a MAR index ranging from 0,43 to 0,71. All the *P. aeruginosa* isolates had a (TTC-CZ-CAZ-CTX-KF-CX) multi-resistance phenotype.

**TABLE 6**
**Antimicrobial susceptibility testing for *P. aeruginosa* strains**

| Antibiotic agent | Number (%) of isolates | | |
|---|---|---|---|
| | Susceptible | Intermediate | Resistance |
| Gentamicin | 6 (85,72%) | 1 (14,28%) | 0 (0%) |
| Amikacin | 1 (14,28%) | 0 (0%) | 6 (85,72%) |
| Ofloxacin | 4 (57,14%) | 3 (42,86%) | 0 (0%) |
| Ciprofloxacin | 7 (100%) | 0 (0%) | 0 (0%) |
| Cefazolin | 0 (0%) | 0 (0%) | 7 (100%) |
| Ticarcillin-clavulanic acid | 0 (0%) | 0 (0%) | 7 (100%) |
| Ceftazidime | 0 (0%) | 0 (0%) | 7 (100%) |
| Piperacillin-tazobactam | 7 (100%) | 0 (0%) | 0 (0%) |
| Piperacillin | 7 (100%) | 0 (0%) | 0 (0%) |
| Tobramycin | 2 (28,58%) | 0 (0%) | 5 (71,42%) |
| Cefotaxime | 0 (0%) | 0 (0%) | 7 (100%) |
| Aztreonam | 6 (85,72%) | 1 (14,28%) | 0 (0%) |
| Cephalothin | 0 (0%) | 0 (0%) | 7 (100%) |
| Cefoxitin | 0 (0%) | 0 (0%) | 7 (100%) |

**TABLE 7**
**Multidrug resistance patterns of *P. aeruginosa* strains**

| ***Pseudomonas aeruginosa* strains** | **Antibiotic susceptibility patterns** | **N° of P.a isolates** | **N° (%) of P.a isolates 7 (100%)** | **N° of antibiotics tested (n=14)** | **MAR index** |
|---|---|---|---|---|---|
| E1 | AK-OF-CZ-TTC-CAZ-TOB-CTX-KF-CX | 1 | 1 (14,28%) | **9** | **0,64** |
| E2 | CZ-TTC-CAZ-CTX-KF-CX | 1 | 1 (14,28%) | 6 | 0,43 |
| E3 | CN-AK-OF-CZ-TTC-CAZ-TOB-CTX-KF-CX | 1 | 1 (14,28%) | **10** | **0,71** |
| E9 ; E19 | AK-CZ-TTC-CAZ-TOB-CTX-KF-CX | 2 | 2 (28,58%) | 8 | 0,57 |
| E12 | AK-CZ-TTC-CAZ-ATM-CTX-KF-CX | 1 | 1 (14,28%) | 8 | 0,57 |
| E17 | AK-OF-CZ-TTC-CAZ-CTX-KF-CX | 1 | 1 (14,28%) | 8 | 0,57 |
| P. *aeruginosa* ATCC 27853 | CZ-TTC-CAZ-CTX-KF-CX | / | / | 6 | 0,43 |

**E:** Bacterial strains; **P.a:** *Pseudomonas aeruginosa*; **ATCC:** American Type Culture Collection; **CX:** Cefoxitin; **CTX:** Cefotaxime; **CAZ:** Ceftazidime; **AK:** Amikacin; **CN:** Gentamicin; **ATM:** Aztreonam; **CZ:** Cefazolin; **KF:** Cephlothin; **OF:** Ofloxacin; **TTC:** Ticarcillin-clavulanic acid; **TOB:** Tobramycin.

**TABLE 8**
**ESBL tests for *P. aeruginosa* strains**

| **Bacterial strains** | **ESBL Tests** | | | | | |
|---|---|---|---|---|---|---|
| | **Synergy test** | | **DDST** | | **Phenotypic tests on a cloxacillin-containing agar** | |
| | -ve | +ve | -ve | +ve | -ve | +ve |
| *P. aeruginosa* | 7(100%) | 0(0%) | 7(100%) | 0(0%) | 7 (100%) | 0(0%) |
| | **Conclusion** | | | | | |
| | Non-ESBL-producing *P. aeruginosa* strains | | | | Cephalosporinase-producing *P. aeruginosa* | |

**DDST:** Double-disc synergy test; **ESBL:** Extended-spectrum beta-lactamase; **+ve:** Positive test; **-ve:** Negative test

**Phenotypic detection of ESBL.** Based on the high resistance of *P. aeruginosa* strains towards the cephalosporin antibiotics class, ESBL tests were carried out.

The ESBL test results are shown in Table 8 and Figure 5 below.

According to the ESBL test results, all the isolated *P. aeruginosa* strains were not ESBL producers, but rather cephalosporinase-producing isolates.

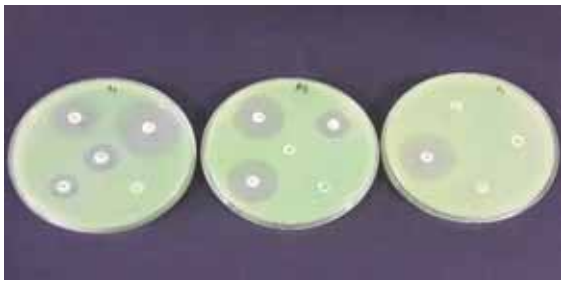


**FIGURE 4**
**AST for *Pseudomonas aeruginosa* (E3)**

**Biofilm formation.** The TM method results indicate that all *P. aeruginosa* strains were able to form biofilms, which was revealed by the adhesion of the bacterial mass to the bottoms and surfaces of the polystyrene tube as shown in Figure 6 below.

## DISCUSSION

In this study, 10 water samples taken from the feed water tank of a dairy unit located in Bechar were analyzed to isolate *Pseudomonas* spp, and then to determine their virulence factors, namely the hemolytic power, pigments production, biofilm formation, and antibiotic resistance pattern.

The bacteriological analysis showed that 7/10 (*i.e.* 70%) of the water samples were contaminated with *Pseudomonas* spp, and through identification tests based on microscopic, biochemical, and metabolic characteristics, and API test system, it confirmed that the isolated strains belong to the *P. aeruginosa* species. These findings are consistent with those of Amato et al. [30], the API-20E multitest system was found to be able to identify *Pseudomonas aeruginosa* isolates. Whereas other tests, such as the acetamide reaction, growth at 42°C, and the oxidative glucose test, can help to identify *P. aeruginosa* species.

The World Health Organization, EU legislation, and Algerian water regulations (JORA) [31] require no *P. aeruginosa* can be detected in 250 mL of drinking water, and limit the amount of *P. aeruginosa* in drinking water to the maximum possible number, less than 3 CFU/L [8]. Based on the results obtained, the quality of the studied feed water tank is questionable.

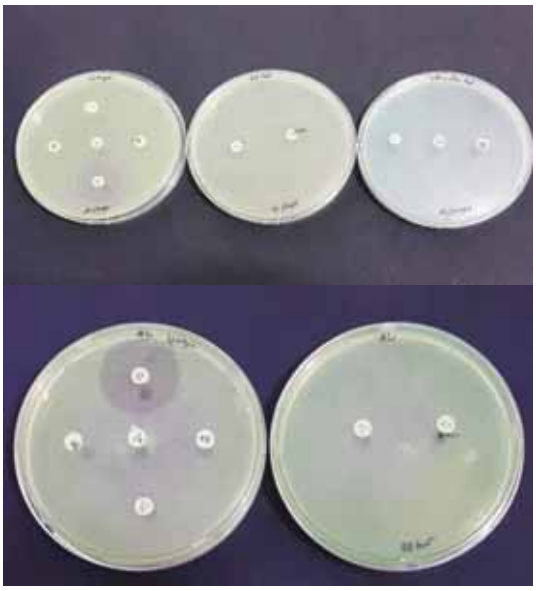


**FIGURE 5**
**Phenotypic detection of ESBL**

The S4, S5, and S6 samples (*i.e.* 30%) of the water samples analyzed were *Pseudomonas* spp-free. The collection of these three samples coincides with the month of Ramadan (the ninth month of the Islamic calendar, a month of fasting for Muslims) known for its high production of reconstituted milk at the rate of 40000 L per day as well as that of lemonade. This production requires a daily filling of the water tank, which is almost exhausted on the same day.

This is probably due either to the disinfection operation of the water tank in preparation for Ramadan, and also due to the daily filling of the feed water tank, as water stagnation can be one of the factors affecting the viable bacteria load and biofilm microbial communities in drinking water [32, 33].

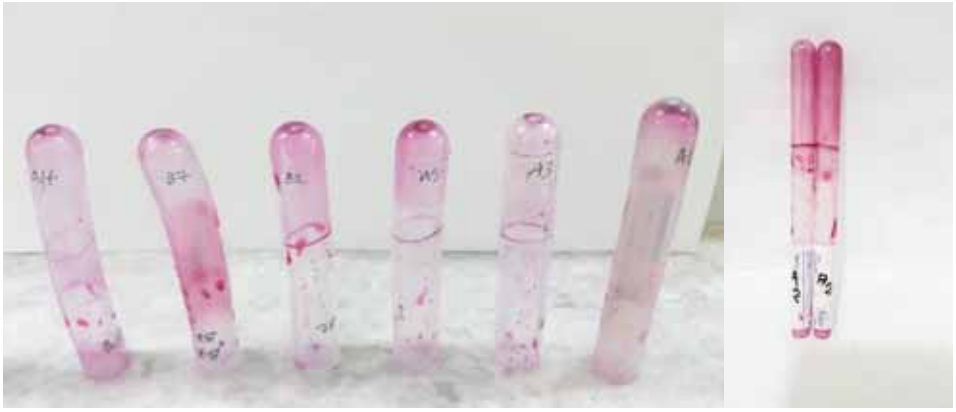


**FIGURE 6**
**Tube method (TM) for the detection of biofilm formation**

Although no waterborne outbreaks have been reported due to water contamination with *Pseudomonas* spp compared with other pathogenic microorganisms such as *Vibrio cholera*, *Escherichia coli* O157:H7, etc., the presence of *P. auerοginosa* is unacceptable because it is an opportunistic microorganism, capable inducing infections in some vulnerable groups such as children, the elderly, and immunocompromised people [34].

Besides, *P. aeruginosa* is often monitored as an indicator of other bacterial contaminants of fecal origin [34], which is consistent with studies previously conducted on the bacteriological quality of the feed water tank of the same dairy unit with the presence of total and fecal coliforms contamination [35].

According to Li et al. [8], and Hartemann [36] the pollution of drinking water by *P. aeruginosa* occurs from time to time, due to many factors such as imperfect disinfection, cross-contamination of source water, water tanks, water pipelines, filtration in the water production process. Moreover, high temperatures and aeration enhance the growth of *P. aeruginosa,* which can resist low concentrations of residual free chlorine. At free chlorine concentrations below 0,3mg/L, the prevalence of *P. aeruginosa* is high and vice-versa [5, 35-38].

A biofilm is defined as a structured organization of microorganisms in a protective and nutritive polysaccharide-protein matrix, which adheres to a surface [39, 40].

The tube method (TM) showed that the *P. aeruginosa* isolates can form biofilms that confer this opportunistic pathogen's high capacity for adaptation, survival, and resistance in the environment [41, 42]. According to Tremblay et al. [43], the presence of biofilms on surfaces can affect the effectiveness of the disinfection protocol. Moreover, *P. aeruginosa* can be particularly difficult to eliminate due to its combinatory antibiotic resistance, multifactorial virulence, and high adaptability [44].

Several research studies have reported the ability of this species to colonize the water distribution system by forming biofilms, which gives it resistance to disinfectants and can represent a health risk at points of use [4, 32, 41, 43, 45, 46].

Pyocyanin and pyoverdine pigments are one of the important virulence factors among many virulence factors of *P. aeruginosa* [47]. All the *P. aeruginosa* isolates are pigment-producing. Finlayson and Brown [48] report that pigmented strains of *P. aeruginosa* are highly virulent than non-pigmented strains. This conclusion was enhanced by the study results of Macin and Akyon [21] who indicate that phenotypic and genotypic virulence factors may be related to pigment production in *P. aeruginosa*, which pigment-producing *P. aeruginosa* have more genes encoding exotoxins such as hlB, exoS, and exoY virulence gene compared to non-pigmented strains.

All isolated *P. aeruginosa* strains showed β-hemolysis. This is reported by several studies [6, 19, 21, 49-53] in which *P. aeruginosa* strains secrete a heat-sensitive enzyme, phospholipase C endowed with hemolytic activity.

The isolated strains showed a resistance rate of 100% to cefazolin, ticarcillin-clavulanic acid, ceftazidime, cefotaxime, cephalothin, and cefoxitin, and at a lower rate of resistance, *i.e*. 86 and 57% for amikacin and tobramycin, respectively. However, ciprofloxacin, ofloxacin, piperacillin, piperacillin-tazobactam, and gentamicin seemed to be the most active molecules, which differs from the results obtained by Maclean et al. [54], in which the isolated *P. aeruginosa* strains were 100% resistant to ciprofloxacin, ofloxacin, piperacillin-tazobactam, and imipenem.

Several studies have shown a high resistance rate of *P. aeruginosa* to many antibiotics, namely ceftazidime, cefepime, amikacin, tobramycin, penicillin, amoxicillin, and cefotaxime [8, 19, 21, 54, 55].

However, these results were different from those obtained by Papapetropoulou et al. [56] where no resistant strains of *P. aeruginosa* and *P. stutzeri* were reported against any of the newer antibiotics tested, except for fluoroquinolones.

Mérens et al. [57], and Moradali et al. [42] reported that the development of β-lactam resistance in *Pseudomonas aeruginosa* results from mutations leading to the expression of cephalosporinase AmpC, efflux systems, decreased permeability, and acquisition of resistance genes, whereas Eyquem et al. [58], and Veyssiere [59] showed that *P. aeruginosa* resistance may be due to non-enzymatic mechanisms such as impermeability to β-lactams.

The MAR index of *P. aeruginosa* isolates showed values ranging from 0,43 to 0,71, corresponding to resistance against 6 to 10 of the 14 tested antibiotics. All the *P. aeruginosa* isolates were classified as multidrug-resistant (MDR). This rate is high compared to that obtained by Roulova et al. [55] (100% against 28,8%). Hocquet et al. [60] reported that antimicrobial resistance should now be viewed as an environmental pollutant, due to various effluent discharges into sewage networks with no treatment process for hospital or industrial wastewater that exert a continuous selective pressure.

*P. aeruginosa* has intrinsic resistance to many antibiotics [21, 55]. However, acquired resistance in this organism is due to chromosomal mutations and the acquisition of resistance genes [60, 61]. For this, there are two main mechanisms;

1st. Inducible chromosomal AmpC β-lactamase, include notably the ESBL, the carbapenemases that hydrolyze most β-lactams, aminoglycoside-modifying enzymes (Tobramycin, Amikacin), and 16S rRNA methylases that provide high-level pan-aminoglycoside resistance ;

2nd. Through several efflux pump systems (MexAB- OprM, MexXY- OprM/A, MexCD- OprJ, and MexEF- OprN) that can simultaneously compromise most β-lactams, leaving only the aminoglycosides and imipenem [21, 28, 61, 62, 63].

Through three ESBL phenotypic tests, the obtained results showed that these isolates were not ESBL producers, but the increase in the zone of inhibition around the 3rd generation cephalosporins discs used in the phenotypic test on a cloxacillin-containing agar may suggest the production of cephalosporinase. Livermore [63] reported that *Pseudomonas aeruginosa* carries multidrug-resistant plasmids and has resistance to cephalosporins. Furthermore, Hamze et al. [64] showed that *Pseudomonas putida* strains isolated from several wells in Akkar (North of Lebanon) used for human consumption and irrigation were cephalosporinase hyper-producing isolates.

**Study limitations.** The study was focused on water samples taken from the feed water tank of the dairy unit, so the data need to be further verified by analyzing the pipe water that comes from the borehole before their storage. Further studies aimed at characterizing resistance genes in *P. aeruginosa* isolated from drinkable water in distribution systems are required.

## CONCLUSION

This study made it possible to detect and isolate *Pseudomonas* spp from the feed water tank, and to study some of their virulence factors.

The obtained results showed a contamination of 7/10, *i.e.* a rate of 70% of the analyzed water samples with *Pseudomonas* spp. The isolates belong to the *P. aeruginosa* species, which are characterized by their ability to hydrolyze proteins, to produce pigmentations (pycocyanin and pyoverdine). Moreover, the isolates showed β-hemolysis and can form biofilms.

*P. aeruginosa* isolates were resistant to the most relevant antipseudomonal drugs such as the cephalosporins and aminoglycosides classes, except for fluoroquinolones (ciprofloxacin and ofloxacin), which could serve as potential vectors for the spread of antibiotic resistance.

For this, the following recommendations can be suggested :

- Develop and protect water tanks, water points, and catchment areas to avoid pollution risks ;
- Collect, transport, and store water in satisfactory sanitary conditions ;
- Monitoring water quality by carrying out physicochemical and bacteriological analysis periodically ;
- Following a strict cleaning and disinfecting plan for the water tank to avoid any microbial contamination or the formation of biofilms ;
- Further investigation for other resistance genes of bacterial isolates found in water is needed.

## LIST OF ABBREVIATIONS

Double-disc synergy test (DDST)
Extended-spectrum beta-lactamase (ESBL)
Multidrug-resistant (MDR)
Pandrug-resistant *Pseudomonas aeruginosa* (PDRPA)
Pyocyanin (PYO)
Pyoverdine (PVD)
Tube method (TM)

## ACKNOWLEDGEMENT

The author sincerely thanks the private dairy unit directorate for allowing him to collect water samples.

## DECLARATIONS

**Ethics approval and consent to participate.** This study complies with the ethical requirements given by the National Health Sciences Ethics Council.

**Human and animal rights.** Not applicable.

**Consent for publication.** Not applicable.

**Availability of data and materials.** The datasets used and/or analyzed during the current study are available from the corresponding author on request.

**Standard of reporting.** STROBE guidelines were followed.

## REFERENCES

[1] Brémaud, C., Thibault, J., Ulrich, E., Randi, F. (2012) Environnement, santé, alimentation. Ed. Educagri, France. 203pp.
[2] Health Canada (2022) Guidance on monitoring the biological stability of drinking water in distribution systems. Canada. 88pp.
[3] Xu, J., Lan, X., Cheng, J., Zhou, X. (2022) Facil synthesis of g-$C_3N_4$/$Ag_2C_2O_4$ heterojunction composite membrane with efficient visible light photocatalytic activity for water disinfection. Chemosphere. 295, 133841.

[4] Bourafa, N, Boutefnouchet N. (2008) Evaluation du risque de contamination des réseaux potables dans les établissements de santé par *Pseudomonas aeruginosa* dans la ville de Annaba-Algérie. Sci. Technol. A. 27(B): 91−96.
[5] Mena, K.D., Gerba, C.P. (2009) Risk assessment of *Pseudomonas aeruginosa* in water. Rev. Environ. Contam. Toxicol. 201, 71−115.
[6] Ezeador, C.O., Ejikeugwu, P.C., Ushie, S.N., Agbakoba, N.R. (2020) Isolation, identification and prevalence of *Pseudomonas aeruginosa* from clinical and environmental sources in Onitsha Metropolis, Anambra State. Eur. J. Med. Health Sci. 2(2), 1−5.
[7] Bricha, S., Ounine, K., Oulkheir, S., El Haloui, N.E., Attarassi, B. (2009) Virulence factors and epidemiology related to *Pseudomonas aeruginosa*. Rev. Tunis. Infect. 2, 7−14.
[8] Li, X., Gu, N., Huang, T.Y., Zhong, F., Peng, G. (2022) *Pseudomonas aeruginosa*: A typical biofilm forming pathogen and an emerging but underestimated pathogen in food processing. Front Microbiol. 13, 1114199.
[9] Wang, C.Y., Jerng, J.S., Cheng, K.S., Lee, L.N., Yu, J.C., Hsueh, P.R., Yang, P.C. (2006) Pandrug-resistant *Pseudomonas aeruginosa* among hospitalised patients: clinical features, risk-factors and outcomes. Clin. Microbiol. Infect. 12(1), 63−68.
[10] Elsen, S., Huber, P., Bouillot, S., Couté, Y., Fournier, P., Dubois, Y., Timsit, J.F., Maurin, M., Attrée, I. (2014) Type III secretion negative clinical strain of *Pseudomonas aeruginosa* employs a two-partner secreted exolysin to induce hemorrhagic pneumonia. Cell. Host. Microbe. 15(2), 164−176.
[11] D'Agata, E. (2015) *Pseudomonas aeruginosa* and other *Pseudomonas* species. In: Bennett, J.E., Blaser, M.J., Dolin, R. (eds). Mandell, Douglas, and Bennett's principales and practice of infectious diseases. 8th Edition, Ed. Elseviers, PA-USA. 2, 2518−2531.
[12] Benyagoub, E., Nabbou, N., Bendada, F. (2018) Characterization of microbial risk level of liquid waste from a private dairy factory in Bechar (Southwest of Algeria). Indian J. Dairy Sci. 71(5), 524−529.
[13] Benyagoub, E. (2019) Investment challenges in milk sector (Case of a dairy factory in southwest of Algeria): Development prospects. Asian J. Dairy Food Res. 38(1), 22−27.
[14] Rodier, J., Legube, B., Merlet, N. (2009) Water analysis (l'Analyse de l'eau). 9th Ed. Dunod, Paris-France. 1579pp.
[15] ISO 16266. (2006) Water Quality- Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration.
[16] Tille, M.P. (2018) Bailey & Scott's diagnostic microbiology, 14th Ed. Elsevier. 1136pp.
[17] Guillot, G., Névot, A., Thieulin, G. (1950) Searched in natural milk for certain pathogenic bacteria for humans (Recherché, dans le lait en nature de certaines bactéries pathogènes pour l'homme). Le lait. 30(297), 337−349.
[18] Waller, J.R., Hodel, S.L., Almquist, D.B. (1985) An evaluation of five techniques used to detect desoxyribonuclease production by bacteria. J. Microbiol. Methods. 3(5−6), 303−309.
[19] Macin, S., Akarca, M., Sener, B., Akyon, Y. (2017) Comparison of virulence factors and antibiotic resistance of *Pseudomoas aeruginosa* strains isolated from patients with and without cyctic fibrosis. Rev. Romana Med. Lab. 25(4): 327−334.
[20] Claude, R., Martine, K. (1995) Laboratory methods for the identification of strictly aerobic Gram-negative bacilli: *Pseudomonas, Alcaligenes, Flavobacterium, Acinetobacter, Brucella, Bordetella.* 2nd Edition, Ed. Institut Pasteur, Paris-France. 332pp.
[21] Macin, S., Akyon, Y. (2017) Phenotypic and genotypic virulence factors in *Pseudomonas aeruginosa* strains according to pigment peresence. Acta Med. Mediterr. 33(6), 1033−1038.
[22] Christensen, G.D., Simpson, W.A., Younger, J.J, Baddour, L.M., Barrett, F.F, Melton, D.M., Beachey, E.H. (1985) Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. 22, 996−1006.
[23] Kirmusaoglu, S. (2019) The methods for detection of biofilm and screening antibiofilm activity of agents. Ed. Intechopen. 152pp.
[24] CLSI. (2018) Performance standards for antimicrobial disk susceptibility testing M100. 28th Edition, Wayne Pennsylvania 19087-USA. 258pp.
[25] Nyandjou, Y.M.C., Yakubu, S.E., Abdullahi, I.O., Machido D.A. (2019) Multidrug resistance patterns and multiple antibiotic resistance index of *Salmonella* species isolated from waste dumps in Zaria Metropolis, Nigeria. J. Appl. Environ. Manage. 23(1), 41−46.
[26] Alkhudhairy, MK, Saki M, Seyed-Mohammadi S, Jomehzadeh N, Khoshnood S, Moradzadeh M, Yazdansetad S. (2019) Integron frequency of *Escherichia coli* strains from patients with urinary tract infection in Southwest of Iran. J. Acute Dis. 8(3), 113−117.
[27] Ayandele, A.A., Oladipo, E.K., Oyebisi, O., Kaka, M.O. (2020) Prevalence of multi-antibiotic resistant *Escherichia coli* and *Klebsiella* species obtained from a tertiary medical institution in Oyo state, Nigeria. Qatar Med. J. 2020(1), 9.

[28] Benyagoub, E., Alkhudhairy, M.K., Benchaib, S.M., Zaalan, A., Mekhfi, Y., Teyebi, N., Razni, D., Bendada, F. (2021) Isolation frequency of uropathogenic strains and search for ESBL producing *Enterobacteriaceae* isolated from patients with UTI in Bechar (Algeria). Antiinfect. Agents. 19(3), 303−316.

[29] Drieux, L., Brossier, F., Sougakoff, W., Jarlier, V. (2008) Phenotypic detection of extended-spectrum β-lactamase production in *Enterobacteriaceae*: review and bench guide. Clin. Microbiol. Infect. 14(1), 90−103.

[30] Amato, S., Vanik, J., Kocka, F.E. (1980) Identification of *Pseudomonas aeruginosa* with the API-20E system. Can. J. Microbiol. 26(4), 554−555.

[31] Ministry of Commerce. (2017) Interministerial order of 2 Moharram 1438 corresponding to October 4, 2016 setting the microbiological criteria for foodstuffs (Arrêté interministériel du 2 Moharram 1438 correspondant au 4 octobre 2016 fixant les critères microbiologiques des denrées alimentaires). J.O.R.A. n.39, 1−32.

[32] Bédard, É. (2015) Operational factors influencing occurrence and risk exposure to *Pseudomonas aeruginosa* and *Legionella peumophila* from hospital water systems. PhD thesis, Polytechnique School of Montreal, France.

[33] Goraj, W., Pytlak, A., Kowalska, B., Kowalski, D., Grzadziel, J., Szafranek-Nakonieczna, A., Galazka, A., Stepniewska, Z., Stepniewski, W. (2021) Influence of pipe material on biofilm microbial communities found in drinking waters supply system. Environ. Res. 196, 110433.

[34] Kouchesfahani, M.M., Alimohammadi, M., Nabizadeh Nodehi, R., Aslani, H., Rezaie, S., Asadian, S. (2015) *Pseudomonas aeruginosa* and Heterotrophic bacteria count in bottled waters in Iran. Iran J. Public Health. 44(11), 1514−1519. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4703231/ (Accessed date: 25/06/2023)

[35] Benyagoub, E. (2023) Softened water and steam boiler water condensate quality used in the milk production line of a private dairy unit located in Bechar province (South-West of Algeria): Physicochemical and bacteriological analysis. Asian J. Dairy Food Sci. (Article *in press*).

[36] Hartemann, P. (2022) Maitrise du risque infectieux lié à l'eau dans les établissements recevant du public: limites de la désinfection des réseaux intérieurs de distribution. Hygiène. 30(3), 207−214.

[37] Price, D., Ahearn, D.G. (1988) Incidence and persistence of *Pseudomonas aeruginosa* in whirlpools. J. Clin. Microbiol. 26(9), 1650−1654.

[38] Hartemann, P., Coornoyer, B., Dubrou, S., Gassilloud, B., Joyeux, M., Leriche, F., Lévi, Y., Monfort, P. (2010) Assessment of health risks associated with exposure by ingesting *Pseudomonas* in water intended for human consumption (excluding bottled water) (Évaluation des risques sanitaires liés à l'exposition par ingestion de Pseudomonades dans les eaux destinées à la consommation humaine (hors eaux conditionnées). Report of the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) 85pp. Available from: https://www.anses.fr/fr/system/files/EAUX2008sa0117.pdf (Accessed date: 28/06/2023)

[39] Husson, G.P., Lecso, M., Hui, F., Lédion, J. (2012) Research of mineral matters and bacteria of the biofilm in the pipe of drinkable water. Tech. Sci. Methodes. 10, 32−41.

[40] Benyagoub, E. (2023) Methicillin, β-lactams, and clindamycin resistance profiles of *Staphylococcus aureus* strains isolated from patients with UTI in Bechar province (Algeria). Antiinfect. Agents. 21, e220823220161.

[41] Pessereau, C. (2015) Study of biotic and abiotic factors that control the implementation of *Pseudomonas aeruginosa* biofilms in thermal water distribution networks (Étude de facteurs biotiques et abiotiques qui contrôlent l'implantation des biofilms de *Pseudomonas aeruginosa* dans les réseaux de distribution d'eau thermal). PhD thesis, Nantes Angers Le Mans University, France.

[42] Moradali, M.F., Ghods, S., Rehm, B.H.A. (2017) *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and resistance. Frot. Cell. Infect. Microbiol. 7, 39.

[43] Tremblay, Y.D.N., Hathroubi, S., Jacques, M. (2014) Les biofilms bactériens: leur importance en santé animale et en santé publique. Can. J. Vet Res. 78(2), 110−116. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3962273/ (Accessed date: 30/06/2023)

[44] Reig, S., Le Gouellec, A., Bleves, S. (2022) What is new in the anti-*Pseudomonas aeruginosa* clinical development pipeline since the 2017 WHO alert ? Front Cell. Infect. Microbiol. 12, 909731.

[45] Poitelon, J.B., Joyeux, M., Welté, B., Duguet, J.P., DuBow, M.S. (2011) The drinking water distribution network : A complex ecosystem related to public health issues. J. Water Sci. 24(4), 383−418.

[46] Lamache, A., Doghri, I., Jacques, M., Boujenah-Haroun, S. (2020) Characterization of bacterial biofilms isolated from drinking water distribution system in Southeastern Algeria. J. Water Sci. 32(4), 447−461.

[47] Kamal, M.A., Pambuk, C.I.A., Husein, A.S. (2016) The virulence of pigmented and non-pigmented *Pseudomonas aeruginosa* in mice with antibiotics susceptibility. Int. J. Vaccines Vaccin. 3(1), 12−14.
[48] Finlayson, E.A., Brown, P.D. (2011) Comparison of antibiotic and virulence factors in pigmented and non-pigmented *Pseudomonas aeruginosa*. West Indian Med. J. 60(1), 24−32. https://pubmed.ncbi.nlm.nih.gov/21809707/ (Accessed date: 13/07/2023)
[49] Johnson, M.K., Boese-Marrazzo, D. (1980) Production and properties of heat-stable extracellular hemolysin from *Pseudomonas aeruginosa*. Infect. Immun. 29(3), 1028−1033.
[50] Ostroff, R.M., Vasil, A.I., Vasil, M.L. (1990) Molecular comparison of a nonhemolytic and a hemolytic phospholipase C from *Pseudomonas aeruginosa*. J. Bacteriol. 172(10), 5915−5923.
[51] Okino, N., Ito, M. (2007) Ceramidase enhances phospholipase C-induced hemolysis by *Pseudomonas aeruginosa*. J. Biol. Chem. 282(9), 6021−6030.
[52] Rossignol, G. (2007) Study of virulence factors of a hospital strain of *Pseudomonas aeruginosa*: hemolytic activity and phenotypic variation (Contribution à l'étude de facteurs de virulence d'une souche hospitalière de *Pseudomonas fluorescens*: activité hémolytique et variation phénotypique). PhD thesis, University of Rouen, France.
[53] Hossain, G., Saha, S., Rahman, M., Singha, J., Mamun, A. (2013) Isolation, identification and antibiogram study of *Pseudomonas aeruginosa* from cattle in Bangladesh. J. Vet. Adv. 3(7), 180−185.
[54] Maclean, K., Njamo, F.J.P., Serepa-Dlamini, M.H., Kondiah, K., Green, E. (2022) Antimicrobial susceptibility profiles among *Pseudomonas aeruginosa* isolated from professional SCUBA divers with otitis externa, swimming pools and the ocean at a diving operation in South Africa. Pathog. 11(1), 91. PMID: 35056039.
[55] Roulova, N., Motkova, P., Brozkova, I., Pejchalova, M. (2022) Antibiotic resistance of *Pseudomonas aeruginosa* isolated from hospital wastewater in the Czech republic. J. Water Health. 20(4), 692−701.
[56] Papapetropoulou, M., Iliopoulou, J., Rodopoulou, G., Detorakis, J., Paniara, O. (2016) Occurrence and antibiotic-resistance of *Pseudomonas* species isolated from drinking water in Southern Greece. J. Chemother. 6(2), 111−116.
[57] Mérens, A., Dalacour, H., Plésiat, P., Cavallo, J.D., Jeannot, K. (2011) *Pseudomonas aeruginosa* and antibiotic resistance. Rev. Francoph. Lab. 2011(435), 49−62.
[58] Eyquem, A., Alouf, J., Montagnier, L. (2000) Treatise on clinical microbiology (Traité de microbiologie Clinique). Ed. Piccin. 238pp.
[59] Veyssiere, A.J. (2019) Antibiotic resistance of the most common bacteria in community infections (La résistance aux antibiotiques des bactéries les plus communément rencontrées dans les infections communautaires). PhD thesis, University of Bordeaux, France.
[60] Hocquet, D., Muller, A., Bertrand, X. (2016) What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. J. Hosp. Infect. 93(4), 395−402.
[61] Poole, K. (2011) *Pseudomonas aeruginosa* : resistance to the max. Front Microbiol. 2, 65.
[62] Ashraf, M.V., Pant, S., Khan, M.A.H., Shah, A.A., Siddiqui, S., Jeridi, M., Alhamdi, H.W.S., Ahmad, S. (2023) Phytochemicals as antimicrobials prospecting Himalayan medicinal plants as source of alternate medicine to combat antimicrobial resistance. Pharmaceuticals. 16, 881.
[63] Livermore, D.M. (2002) Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? Clin. Infect. Dis. 34(5), 634−640.
[64] Hamze, M., Dabboussi, F., Abdou, T., Radwan, M., Awad, Z. (2010) Study of the bacteriological quality and research of multi-drug resistant bacteria in 50 wells in Akkar in the North of Lebanon. Cah. ASEES. 15(1), 45−53.

**Received: 28.08.2023**
**Accepted: 13.09.2023**

## CORRESPONDING AUTHOR

**Elhassan Benyagoub**
Architecture and Environmental Heritage Laboratory (Archipel),
Mohammed Tahri University of Bechar
(08000) Bechar – Algeria

e-mail: benyagoub.elhassan@univ-bechar.dz
benyagoubelhassan@gmail.com

- © by PSP Volume 32– No. 09/2023 pages 3050-3059 Fresenius Environmental Bulletin

# CORRELATION BETWEEN ODOR CONCENTRATION AND MICROBIAL PRESENCE IN WASTE LEACHATE UNDER DIVERSE CONDITIONS

**Zijun He[1,2], Yuanyan Jiang[1,2], Chuan Huang[1,2,*], Kejin Chen[1,2]**

[1]State Key Laboratory of Coal Mine Disaster Dynamics and Control, Chongqing University, Chongqing, 400044, China
[2]College of Environment and Ecology, Chongqing University, Chongqing, 400044, China

## ABSTRACT

Odor emissions from waste staging areas persistently raise concerns because of their marked effects on human well-being. Leachate from domestic waste is a primary contributor to these emissions. This study investigates the environmental factors shaping leachate odor, highlighting the relationship between alterations in microbial communities and odor variations. Our data show that as the moisture content in waste increases, the odor concentration decreases. Conversely, rising temperature and prolonged fermentation amplify the odor intensity. Changes in microbial population density are directly influenced by fermentation conditions and correlate with leachate odor intensity. Importantly, both *Leuconostoc* and *Enterobacteriaceae* have significant associations with increased temperature and odor intensity. Effective management of these microbial populations provides a feasible strategy to reduce odors from domestic waste leachate.

**KEYWORDS:**
Domestic waste, leachate, environmental factors, odor unit, Environmental variables

## INTRODUCTION

Owing to China's accelerated economic expansion and demographic surge, there has been a notable enhancement in the living standards of its populace. This has consequently led to a surge in the production of domestic waste. Consequently, China is confronted with the imminent challenge of managing municipal waste accumulation. In 2019, China reported a total waste generation of approximately 242.06 million tons, a figure that escalated to 248.69 million tons by 2022[1,2]. Municipal waste is customarily amassed and conveyed by waste transfer vehicles to interim storage facilities termed as waste transfer stations. Subsequently, this waste is relocated to disposal stations situated in the peripheries of the city, a procedure that spans approximately 1 to 3 days[3].

During the waste management process, the transfer and disposal of domestic waste can give rise to leachate formation. This poses significant disturbances to the daily lives of residents, primarily due to the offensive odors emanating from inadequate treatment. Factors contributing to leachate production include breaches in garbage bags and the accumulation of residues at the base of waste containers. Such leachate typically originates during the storage, positioning, and landfilling phases of domestic waste management[4].

Leachate is delineated as the liquid that filters through successive strata of waste, driven by gravitational forces. Its genesis can be attributed to three principal sources: (1) intrinsic moisture content within the waste, (2) water produced during the microbial decomposition of organic material in landfills, and (3) water introduced into the waste from external avenues such as precipitation. As leachate percolates through various layers of a landfill, it assimilates a plethora of toxic contaminants, a consequence of complex interactions, biogeochemical transformations, and hydrological responses[5].

Leachate, characterized by its solubility of both inorganic and organic constituents, encompasses an elevated concentration of contaminants. This includes pronounced levels of chemical oxygen demand (COD), sulfate, ammonia nitrogen, inorganic salts, heavy metals, pathogenic entities, recalcitrant humic substances, and persistent micro/nano-sized plastics[6]. These pollutants pose potential environmental risks and challenges in waste management and disposal[7],among them, there are many types of metals[8] and high levels of ammonia and nitrogen[9]. Addressing leachate management poses a substantial challenge owing to its intricate compositional matrix. While extant research has predominantly concentrated on the implications of leachate contamination in soil and groundwater, the repercussions of malodorous emissions during the initial phases of domestic waste leachate decomposition remain relatively uncharted.

Temperature exerts a considerable impact on odor generation from domestic waste. Notably, the odor concentration emanating from the fermentation of domestic waste is discernibly diminished during the summer months in comparison to the winter pe-

riod[10].Temperature plays a crucial role in determining the effectiveness of anaerobic digestion processes[11]. Variations in temperature also have significant impacts on the structure and abundance of microbial populations[12], it also have an important effect on the composition and concentration of malodorous substances in domestic waste[13]. The term "wet-component garbage" denotes the segment of domestic waste characterized by elevated moisture content, encompassing materials like fruit peels and vegetables. Domestic waste, with its intricate compositional matrix, generally possesses considerable moisture content. In China, kitchen waste constitutes the predominant fraction of domestic waste, representing in excess of 50 percent of the aggregate[14]. Wet-component in domestic waste plays a crucial role in facilitating the growth and reproduction of microorganisms[15], Water is essential for microbial populations as it serves as a solvent for their metabolic processes. Microorganisms require water for the solubilization of nutrients necessary for their growth. A portion of the organic matter present in domestic waste is released as liquid, which serves as a nutrient source for microorganisms. Moisture plays a crucial role in dissolving nutrients evenly, influencing the growth and metabolism of microbial populations. It also constitutes an important factor affecting the generation of unpleasant odors from domestic waste[16]. Considering that the fermentation of domestic waste is an ongoing process, it is crucial to investigate the impact of fermentation time on domestic waste leachate[17]. The fermentation time significantly influences the fermentation process of domestic waste leachate [5].

Gao[18]showed that the addition of microbial inoculants can increase the diversity of bacterial communities, promote the presence of functional bacteria, and mitigate gas emissions while enhancing the humification process. However, current research on leachate has focused on the collection process after its formation, neglecting the effect of leachate odor during the initial waste collection phase. Earlier studies didn't deeply explore how the smell from waste water (leachate) is linked to its microbial presence. In our study, we're checking how factors like temperature, the amount of wet items in the waste, and fermentation time influence these microbes. Using gas-detecting tools and examining microbial presence, we hope to better understand the connection between leachate odor and its microbes. Our findings could help us find better ways to manage this odor in waste water. This can help in reducing negative effects on the environment and our health.

## MATERIALS AND METHODS

**Experimental material.** A field survey was conducted across six communities within Chongqing, subsequent to which we undertook the sampling and analytical assessment of domestic waste components. Wet components were epitomized by waste fruit peels, discarded vegetables, and residual rice. For our experimental design, we simulated domestic waste leachate using a specified ratio of kitchen waste, paper waste, and plastic waste. As shown in table1, set at 7:1.5:1.5. Sampling was strategically conducted at 8:00 a.m. on a non-working day, precisely prior to the routine collection and transfer of domestic waste. This exhaustive field study and sampling regimen endowed us with both quantitative data and tangible samples for ensuing experimental evaluations. Our methodology is tailored to engender an enhanced comprehension of the odoriferous emanations intrinsic to domestic waste leachate.

**Experimental design.** To delineate the concentration of odorous compounds in leachate, a systematic experimentation was performed. Configured waste, with varying wet component ratios, underwent fermentation at a controlled temperature of 35℃ for a span of 24 hours. Subsequent to fermentation, the derived leachate was filtered, and this filtrate was exposed to temperature regimes of 15℃, 25℃, and 35℃ for durations of 24, 48, and 72 hours respectively. The odorous concentration within the leachate was quantified using a portable gas detection apparatus.

For the quantification of malodor, the gas detector, model SGA-600B, was employed. A specified gas inlet flow rate of 250 mL/min was maintained, and the detector was initialized for a duration of 60 seconds. To ascertain precision in measurements, an 80-second post-initialization purgation was conducted to negate any lingering odorous compounds.

The efficacy and accuracy of the SGA-600B detector were validated by juxtaposing the detected values with those ascertained through manual olfactory assessment, as delineated by the "Air Quality Standard for Detection of Malodors, Three-Point Comparison Odor Bag Method" (GB 14675-1993). For this procedure, six laboratory personnel were designated as olfactory assessors and engaged in a controlled environment with ambient temperatures ranging from 17℃ to 25℃. Initial olfactory evaluations were based on established dilution metrics. Each evaluation comprised three olfactory sampling bags: two hosting uncontaminated gases and one containing a diluted malodorous sample. The collective olfactory feedback from all assessors determined the presence or absence of odor.

The final quantification of malodor concentrations was executed based on the methodologies elucidated in the "Air Quality Standard for Detection of Malodors, Three-Point Comparison Odor Bag Method" (GB 14675-1993). The resultant values were then benchmarked against those acquired from

the SGA-600B gas detector.

**DNA extraction and measurement.** The leachate collected from the fermentation of domestic waste underwent further analysis through the following steps.

Firstly, the E.Z.N.A. soil DNA kit (Omega Biotek, Norcross, GA, U.S.) was used to extract the microbial community genomic DNA from samples in accordance with the manufacturer's instructions. The DNA extract was examined on a 1% agarose gel, and a NanoDrop 2000 UV–vis spectrophotometer was used to measure the DNA concentration and purity (Thermo Scientific, Wilmington, USA). Using an ABI GeneAmp® 9700 PCR thermocycler and the primer pair 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT), the hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified (ABI, CA, USA). Denaturation at 95°C for three minutes was followed by 27 cycles of denaturing at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, with a single extension at 72°C for 10 mins. The PCR mixtures contained 4 μL of 5 × TransStart FastPfu buffer, 2.5 mM dNTPs, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.4 μL of TransStart FastPfu DNA Polymerase, 10 ng of template DNA, and ddH2O up to 20 μL. Triplicate PCRs were carried out. The AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used to extract the PCR product from a 2% agarose gel, purify it as directed by the manufacturer, and quantify it using a Quantus™ Fluorometer (Promega, USA). Majorbio Bio-Pharm Technology Co. Ltd. used paired-end sequencing on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) using purified amplicons that were pooled in an equimolar way (Shanghai, China).

## RESULTS AND DISCUSSION

**Worker's olfactory values and equipment measurements.** To validate the measurement accuracy of the SGA-600B portable gas detector, a meticulous calibration was conducted. Subsequently, the quantified malodor concentrations from the detector were benchmarked against olfactory assessments performed manually, in accordance with the "Air Quality Standard for Detection of Malodors, Three-Point Comparison Odor Bag Method" (GB 14675-1993). This juxtaposition provided a rigorous

**TABLE 1**
**The percentage of domestic waste**

| Wet-component | Pericarp | Vegetable | Meat | Bone | Plastics | Paper |
|---|---|---|---|---|---|---|
| 30% | 150 | 150 | 100 | 300 | 150 | 150 |
| 50% | 250 | 250 | 50 | 150 | 150 | 150 |
| 70% | 350 | 350 | 0 | 0 | 150 | 150 |

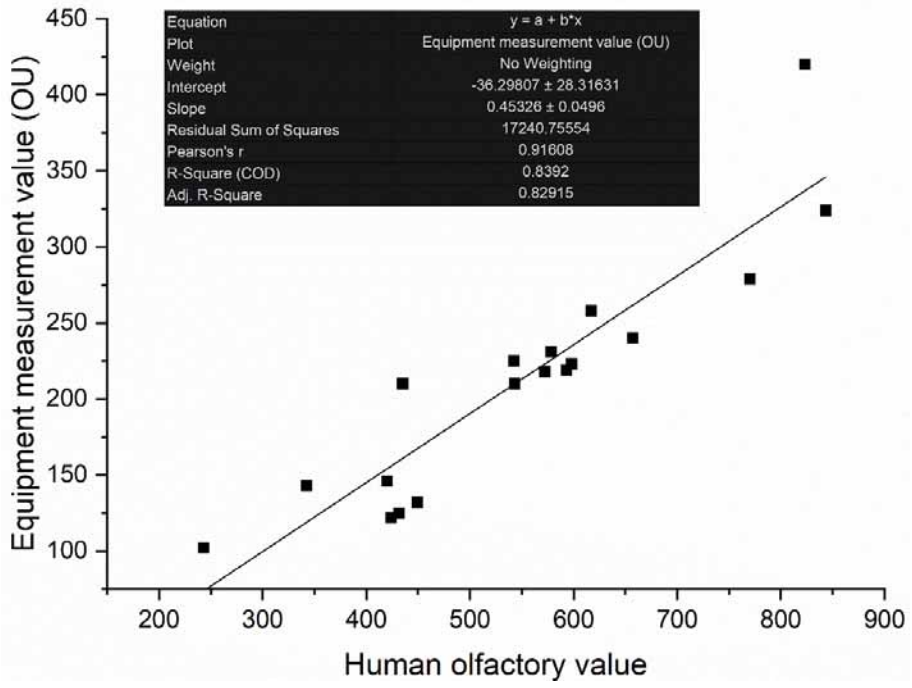



**FIGURE 1**
**The curve of the relationship between the equipment measurement (OU) and the manual olfactory value**
**TABLE 2**

**Alpha diversity of community bacterial community in smelly liquid domestic waste**

| Sample/Estimators | Chao | Shannon | Simpson | Sobs |
|---|---|---|---|---|
| 30-2-48 | 291.10 | 2.88 | 0.20 | 291.00 |
| 70-3-24 | 65.36 | 2.29 | 0.14 | 64.00 |
| 70-2-24 | 75.00 | 2.58 | 0.18 | 75.00 |
| 50-1-72 | 105.60 | 2.95 | 0.10 | 105.00 |
| 70-1-48 | 106.63 | 2.72 | 0.12 | 106.00 |
| 70-2-72 | 94.25 | 2.00 | 0.34 | 94.00 |
| 50-2-24 | 120.00 | 2.31 | 0.26 | 119.00 |
| 70-3-48 | 69.75 | 1.69 | 0.43 | 69.00 |
| 50-1-48 | 145.91 | 3.03 | 0.11 | 145.00 |
| 30-3-48 | 219.36 | 2.41 | 0.31 | 219.00 |
| 70-1-72 | 76.25 | 2.23 | 0.22 | 76.00 |
| 50-3-24 | 89.36 | 1.42 | 0.54 | 88.00 |
| 50-1-24 | 115.40 | 1.95 | 0.41 | 114.00 |
| 30-1-24 | 342.35 | 3.2 | 0.13 | 341 |
| 50-2-48 | 159.27 | 2.45 | 0.26 | 158.00 |
| 50-3-48 | 57.00 | 2.02 | 0.20 | 57.00 |
| 50-3-72 | 75.00 | 2.17 | 0.19 | 75.00 |
| 30-1-72 | 257.19 | 3.15 | 0.10 | 257.00 |
| 30-3-72 | 213.68 | 2.25 | 0.25 | 213.00 |
| 70-2-48 | 94.38 | 2.47 | 0.22 | 94.00 |
| 70-3-72 | 24.50 | 0.42 | 0.87 | 24.00 |
| 30-2-72 | 325.54 | 3.42 | 0.11 | 325.00 |
| 30-2-24 | 306.35 | 3.28 | 0.11 | 305.00 |
| 30-3-24 | 231.09 | 2.97 | 0.16 | 231.00 |
| 70-1-24 | 83.20 | 1.79 | 0.42 | 79.00 |
| 30-1-48 | 230.13 | 2.86 | 0.16 | 230.00 |
| 50-2-72 | 115.00 | 2.11 | 0.28 | 115.00 |

verification of the detector's fidelity, aligning its readings with human evaluative standards. As shown in Figure 1, the fitting function, $y = 0.4533x - 36.298$, with an $R^2$ value of 0.8392, indicates a strong linear relationship between the manual sniffing value and the equipment measurement value. This confirms that the malodor concentration measured by the portable gas detector is reliable and can be utilized for subsequent microbiological analysis.

**Microbial community analysis. As shown in table 2,** the Sobs index denotes the number of observed species, acting as a metric for community richness. Conversely, the Chao index estimates the total potential species count, representing an indicator of species richness. The Shannon index is utilized to characterize community diversity, where an elevated value suggests increased community biodiversity. In contrast, the Simpson index describes community evenness, with a higher value indicating diminished community biodiversity. Together, these indices provide a comprehensive understanding of the Alpha diversity within a specific community.

Alpha diversity, evaluated at the ASV level, revealed variations in microbial diversity among samples exposed to different fermentation conditions. Initially, the Chao and Shannon indices were notably higher for the 30%, 50%, and 70% wet fraction-components. However, with an increase in the wet fraction proportion, the Chao index exhibited a discernible decline. For instance, while the 30% wet fraction exhibited a peak Chao index value of 342.35, the 70% wet fraction presented a minimum value of 24.50, underscoring a significant difference between them.

The Chao index for each sample exhibited a clear pattern: an initial increase followed by a decline with rising temperatures. This observation is likely due to the optimal temperature range favorable for bacterial growth, typically between 25-30°C. Therefore, at a fermentation temperature of 25°C, a larger bacterial population found the conditions favorable, resulting in an increase in species richness. For the sample with a 50% wet-component at this temperature, both species richness and evenness displayed fluctuations, initially declining and then increasing as fermentation proceeded. After 72 hours of fermentation, the Chao index reached 325.54, and the Shannon index peaked at 3.42.

In summary, microbial growth is enhanced by a lower wet-component ratio in waste, an optimal fermentation temperature, and prolonged fermentation. Under ideal conditions for microbial activity, an increase in the concentration of malodors from domestic waste leachate is expected.

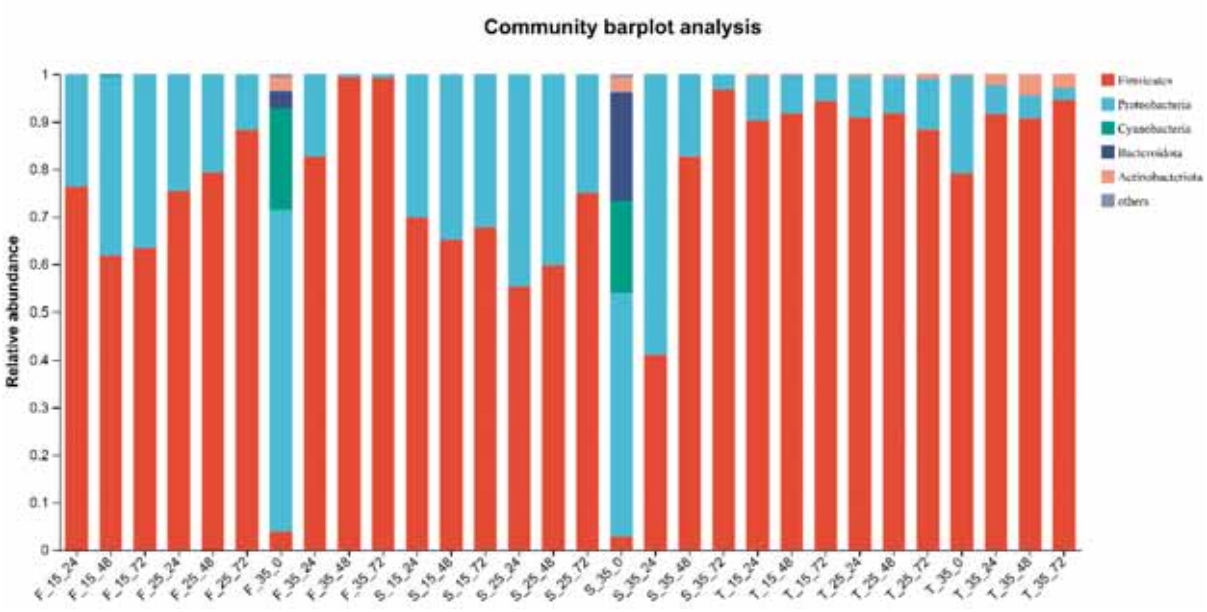



**FIGURE 2**
**Phylum level species composition analysis**

***T-30% wet-component**
***F-50% wet-component**
***S-70% wet-component**
***15/25/35-temperature(°C)**
***24/48/72-time(hour)**

**Analysis of microbial community structure. Community structure at the portal level.** Figure 2 illustrates the species composition at the phylum level. The predominant phyla observed were Firmicutes (ranging from 2.77% to 99.34%), Proteobacteria (0.65% to 67.61%), Cyanobacteria (19.32% to 21.35%), Bacteroidota (3.70% to 22.96%), and Actinobacteriota (2.36% to 4.43%). Among these, Proteobacteria and Firmicutes stood out as particularly abundant. Notably, Firmicutes consistently constituted more than 50% of the microbial composition under each fermentation condition. Contrary to the statement, Firmicutes is not a bacteriophage, but a phylum of bacteria. They exhibit strong resilience to adverse environments and are primarily involved in the decomposition of carbohydrates^19. These observations shed light on the principal phyla found in microbial communities across various fermentation conditions, underscoring the prominence of Proteobacteria and the critical role of Firmicutes in carbohydrate breakdown[19]. The microbial community's composition, featuring Proteobacteria and Firmicutes, points to their integral roles in the breakdown and conversion of organic materials and carbohydrates during the waste fermentation phase. It's noteworthy that the relative abundance of Firmicutes remained relatively stable across varying fermentation temperatures and durations within the 30% wet-component subset. Conversely, for the 50% and 70% wet-component categories, there was a notable increase in Firmicutes' relative abundance as fermentation time extended, particularly at a fermentation temperature of 25°C. Such observations underscore the significance of Firmicutes in carbohydrate decomposition and highlight its adaptability to specific fermentation parameters.

Cyanobacteria, ubiquitously distributed across diverse ecosystems, are renowned for their oxygenic photosynthesis capabilities. Exhibiting adaptability to a range of habitats, from aquatic to arid terrains, they serve a pivotal function in global oxygen generation[20]. At elevated temperatures (35°C and above), a decline in the abundance of Proteobacteria was observed. In contrast, Bacteroidota showed increased prevalence in the absence of fermentation within the 50% and 70% wet-component groups. This observation correlated with the minimal concentrations of malodorous gases detected in domestic waste.

**Horizontal community structure.** Figure 3 presents the bacterial community structure at the genus level in domestic waste leachate under different fermentation conditions. The top five genera identified were *Lactobacillu s*(0.39%~94.58%), *Leuconostoc* (0.32%~68.90%), *Weissella* (0.13%~77.93%), *Streptococcus* (0.09%~57.90%), *unclassified_f__Enterobacteriaceae* (0.08%~31.53%).

*Lactobacillus*, with the highest relative abundance, thrived at an optimal growth temperature of 35°C. In the 50% and 70% wet-component groups, at a fermentation temperature of 35°C, its relative abundance increased with longer fermentation time. Remarkably, in sample S-3-72, *Lactobacillus*

reached a relative abundance of 94.58%. *Lactobacillu* is known for its involvement in carbohydrate fermentation, and its relative abundance reflects the progress of carbohydrate fermentation in the waste leachate. As the fermentation time of domestic waste increased, the microbial community in the leachate underwent differentiation and evolution, adapting to the current substrate and temperature conditions. Higher temperatures accelerated organic matter hydrolysis, leading to a decrease in leachate pH and the gradual development of an acidic environment.

*Leuconostoc*, well-adapted to acidic environments, exhibits rapid reproduction and synthesis of dextran and other saccharides under such conditions. Additionally, *Leuconostoc* can ferment saccharides, leading to the production of various acids[21]. *Leuconostoc* thrives in lower temperatures and increases in abundance with a higher proportion of wet-component in domestic waste leachate. However, its relative abundance decreases with longer fermentation time and higher temperatures. *Leuconostoc* is involved in heterofermentative lactic acid fermentation with citric acid metabolism and is commonly found in vegetables, meats, and fermented foods[22]. This bacterium is a Gram-positive organism that thrives best at temperatures ranging from 22 to 30°C. It exhibits metabolic activity, producing $CO_2$ and a diverse range of compounds.

*Weissella* is a bacterium that can be found in various sources such as vegetables, meat products, and fermented foods. It is also associated with food spoilage[23]. *Weissella* plays a significant role in the fermentation process[24] The relative abundance of *Weissella* increased initially and then decreased as the wet-component increased. In the case of a 50% wet-component and temperature below 35°C, the relative abundance of *Weissella* increased with longer fermentation time. This is because *Weissella* thrives best at temperatures between 30°C and 37°C and within a pH range of 3.0 to 9.5[25]. *Weissella* and *Leuconostoc* are both Gram-positive anaerobic cocci and play crucial roles in the fermentation process.

*Streptococcus*, another Gram-positive bacterium belonging to the Firmicutes phylum, was only observed in domestic waste leachate with a wet-component of 30% and was not detected in samples with wet-component of 50% and 70%[26]. During the initial stages of fermentation, the relative abundance of this bacterium escalated with increasing temperature. Yet, across varying fermentation temperatures, its relative abundance first showed an uptick followed by a decline as the fermentation period extended. Notably, in sample T-3-48, this bacterium exhibited an exceptionally high relative abundance, reaching 57.90%, thereby establishing it as the predominant bacterial presence in that specific setting.

The abundance of *unclassified_f__Enterobacteriaceae* showed a tendency to increase with increasing temperature and proportion of wet-component, and gradually decrease with increasing fermentation time. The optimum growth temperature for *unclassified_f__Enterobacteriaceae* was 37°[27]. The abundance of *unclassified_f__Enterobacteriaceae* decreases with increasing fermentation time and may even become undetectable[28].

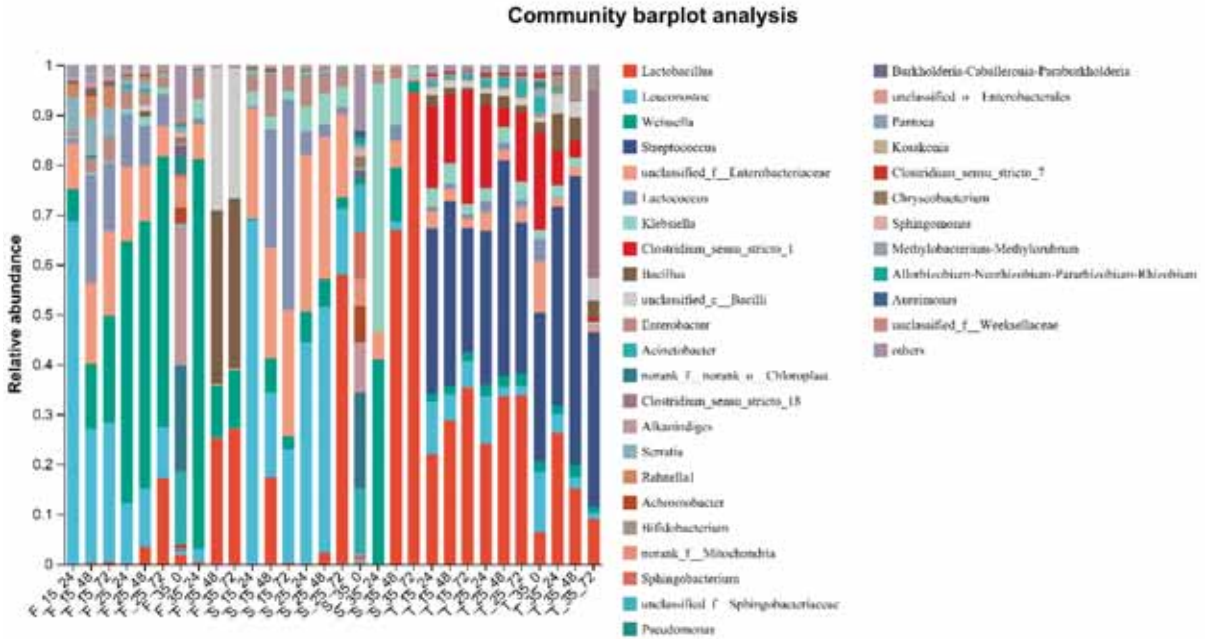



**FIGURE 3**
**Genus level species analysis**

***T-30% wet-component**
***F-50% wet-component**
***S-70% wet-component**
***15/25/35-temperature(°C)**
***24/48/72-time(hour)**

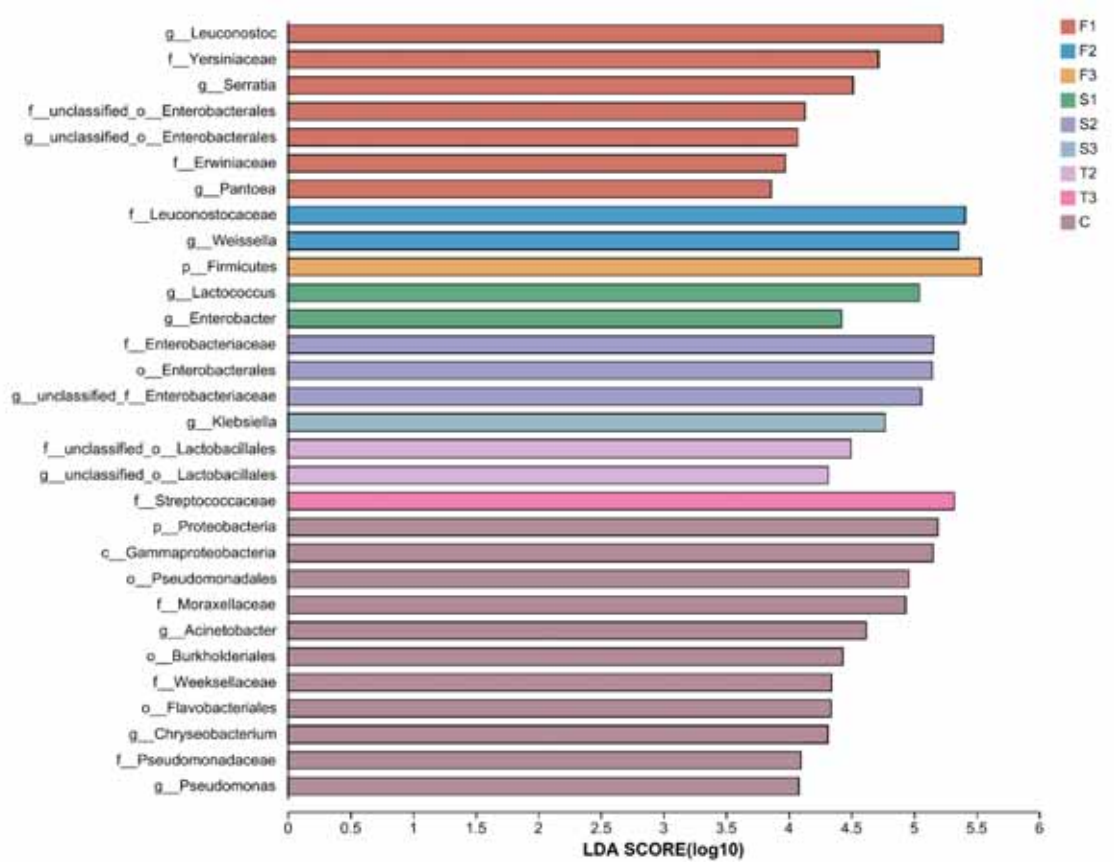



**FIGURE 4**
**LDA discriminant histogram**

***T-30% wet-component**
***F-50% wet-component**
***S-70% wet-component**
***1-15/2-15/3-35-temperature(°C)**

**Analysis of species differences.** A differential species analysis was carried out employing the Linear Discriminant Analysis (LDA) histogram. Figure 4 provides a representation from the phylum to the genus level across varied wet-component proportion groups. This visualization aids in discerning dominant species within distinct environments and pinpointing species variations between samples. The LDA discriminant bar depicted in Figure 4 highlights species with an LDA threshold exceeding 2.0. The LDA score derived from the analysis illustrates the influence of species abundance on the differential effect, with elevated scores denoting a more pronounced impact.

In Figure 4, the proportion of differential indicator species was higher when the wet-component of domestic waste was 50%, with a total of 10 differential species observed at the three fermentation temperatures. The Firmicutes had an LDA score greater than 5.5, indicating that it had the strongest impact on species differences. *Weissella*, a crucial bacterial group in the fermentation process, exhibited various properties such as probiotic, bacteriostatic, and extracellular production capabilities. When the wet-component was 50% and the fermentation temperature was 25℃, *Weissella* obtained an LDA score of 5.36, suggesting that the fermentation environment was favorable for its survival. This finding aligns with th e observed performance of *Weissella* in fruit fermentation *broths*[29].

**Microbial correlation analysis.** The relationship between microbial taxa and environmental parameters was examined using Spearman's correlation coefficient. An analysis focused on the top 10 abundant species at the genus level, evaluating their correlation with factors such as the wet-component ratio, temperature, and odor unit. The outcome of this examination is presented in Figure 5. On this figure, the X-axis denotes environmental variables, while the Y-axis represents the species. The strength and direction of each correlation are signified by the R-values and are color-coded. Statistically significant P-values are annotated: values less than 0.05 are marked with "", those between 0.01 and 0.05 with "", and those less than 0.01 with "". *Streptococcus*, *Clostridium_sensu_stricto_1*, *Bacillus*, and *unclassified_c_Bacilli* showed a significant positive correlation with the wet-component ratio. The abundance of

these bacteria increased as the wet-component ratio increased, suggesting that they thrive in moister environments. *Leuconostoc* exhibited a highly significant positive correlation with temperature and a significant positive correlation with odor unit. This indicates that the abundance of *Leuconostoc* increased as the fermentation temperature rose and was positively associated with malodor production, possibly due to its involvement in souring processes during fermentation[30]. Furthermore, *unclassified_f__Enterobacteriaceae* displayed a highly significant positive correlation with malodor production. This suggests that the abundance of *unclassified_f__Enterobacteriaceae* is positively associated with the production of malodorous compounds. The *Enterobacteriaceae* has been previously found to be positively correlated with odor production in various samples such as sewage, swine feces, cheese, and gastrointestinal tracts[31,32].

## CONCLUSION

At the phylum tier, Firmicutes, Proteobacteria, Cyanobacteria, Bacteroidota, and Actinobacteriota were notably dominant. Specifically, Firmicutes and Proteobacteria showcased the broadest range in abundance.

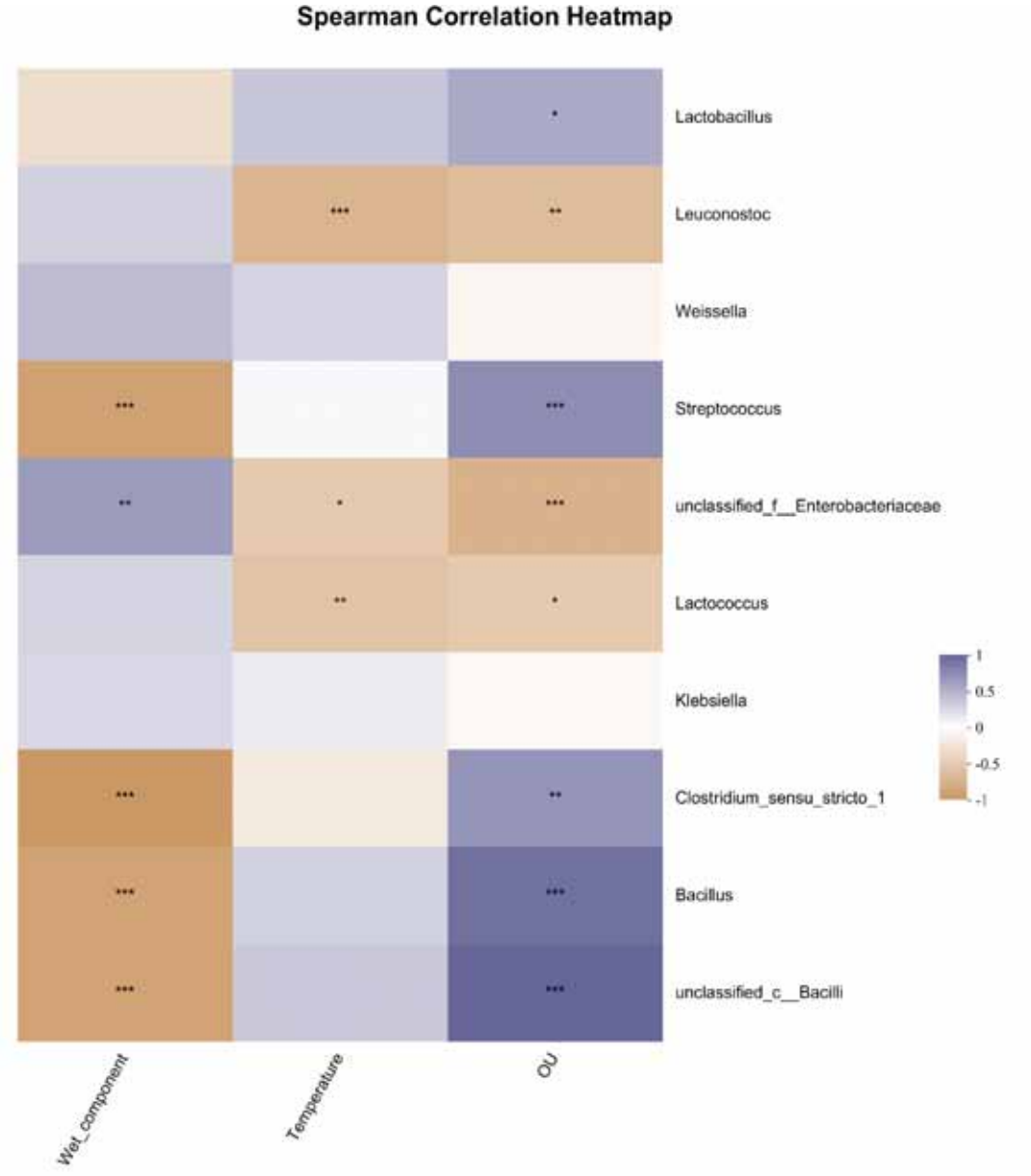



**FIGURE 5**
**Correlation analysis of temperature, wet component ratio, odor concentration and microbial community at genus level**

Zooming into the genus tier, the dominant players were *Lactobacillus*, *Streptococcus, Weissella,* and an *Unclassified_f__Enterobacteriaceae*. Among these, *Lactobacillus* was particularly prominent in terms of relative abundance. Under conditions where the wet-component constituted 30% of domestic waste and fermented for 48 hours, the abundance of *Lactobacillus* surged with rising temperature, but receded when the temperature peaked at 35°C. Meanwhile, *Weissella* exhibited an increase in abundance with rising temperature up to a point, followed by a decline as temperatures transitioned from 15°C to 35°C. Additionally, the abundance of *Weissella* also climbed with an escalating proportion of the wet component and longer fermentation durations.

Different microbial entities displayed varying trends in relative abundance based on distinct fermentation settings. Intriguingly, there was a pronounced correlation between the concentration of odors in leachate and microbial abundance. Specifically, *Leuconostoc* demonstrated a significantly positive correlation with both temperature and odor concentration. Meanwhile, the *Unclassified_f__Enterobacteriaceae* also registered a markedly positive association with odor concentration.

## ACKNOWLEDGEMENTS

This work was supported by the National Key Research and Development Project (2019YFC1906104) and Sharing Fund of Chongqing University's Large-scale Equipment.

**Funding.** National Key Research and Development Project of China (2019YFC1906104)

**Data availability.** Not applicable.

**Code availability.** Not applicable.

**Ethics approval and consent to participate.** Not applicable.

**Consent for publication.** Not applicable.

## REFERENCES

[1] National Bureau of Statistics of the People's Republic of China. (2019) China Statistical Yearbook. China Statistics Press: Beijing. 8-15.

[2] National Bureau of Statistics of the People's Republic of China. (2022) China Statistical Yearbook. China Statistics Press: Beijing. 8-15.

[3] Liu, Y., Chen, J., Yang, H., Wang, J., Zou, K. (2021) Emission of Pollutants and Odor Pollution at Initial Decomposition Stage of Municipal Solid Waste. Research of Environmental Sciences. (In Chinese). 35, 238–245.

[4] Abdel-Shafy, H.I., Ibrahim, A.M., Al-Sulaiman, A.M., Okasha, R.A. (2023) Landfill Leachate: Sources, Nature, Organic Composition, and Treatment: An Environmental Overview. Ain Shams Engineering Journal. 102293.

[5] Abedi, S., Nozarpour, A., Tavakoli, O. (2023) Evaluation of Biogas Production Rate and Leachate Treatment in Landfill Through a Water-Energy Nexus Framework for Integrated Waste Management. Energy Nexus. 100218.

[6] Gripa, E., Daflon, S.D.A., De Almeida, R., Da Fonseca, F.V., Campos, J.C. (2023) Landfill Leachate Treatment by High-Pressure Membranes and Advanced Oxidation Techniques with a Focus on Ecotoxicity and by-Products Management: A Review. Process Safety and Environmental Protection. 173, 747–764.

[7] Ma, S., Zhou, C., Pan, J., Yang, G., Sun, C., Liu, Y., Chen, X., Zhao, Z. (2022) Leachate from Municipal Solid Waste Landfills in a Global Perspective: Characteristics, Influential Factors and Environmental Risks. Journal of Cleaner Production. 333, 130234.

[8] Lee, H., Coulon, F., Beriro, D.J., Wagland, S.T. (2023) Increasing Recovery Opportunities of Metal(Loid)s from Municipal Solid Waste via Landfill Leachate Recirculation. Waste Management. 158, 116–124.

[9] Wu, Y., Wang, B., Chen, G. (2020) Sustainable Landfill Leachate Treatment. Waste Management and Research. 38(10), 1093–1100.

[10] He, P.J., Li, J.C., Lü. F., Zhang, H., Shao, L.M. (2022) Temporal and Spatial Variation in Odor Pollution and Membrane Barrier Effect in Municipal Solid Waste Landfill. Environmental Science. 43, 4506–4512.

[11] Lin, Q., De Vrieze, J., Li, J., Li, X. (2016) Temperature Affects Microbial Abundance, Activity and Interactions in Anaerobic Digestion. Bioresource Technology. 209, 228–236.

[12] Guo, X., Sha, Y., Pu, X., Lu, W., Liu, X., Hu, J., Luo, Y., Wang, J., Li, S., Zhao, Z. (2022) Research Progress on the Effect of Ambient Temperature on Intestinal Microflora of Animals. Acta Veterinaria et Zootechnica Sinica. 53, 2858–2866.

[13] Jiang, Y.; Huang, J.; Luo, W.; Chen, K.; Yu, W.; Zhang, W.; Huang, C.; Yang, J.; Huang, Y. Prediction for Odor Gas Generation from Domestic Waste Based on Machine Learning. Waste Management 2023, 156, 264–271. https://doi.org/10.1016/j.wasman.2022.12.006..

[14] Li, H., Zheng, L., Zhou, H., Sun, Y., Zhang, T., Chen, T., Yang, T., Zhang, B. (2022) Review on Spatial-Temporal Distribution Characteristics of Municipal Solid Waste Components In China. Environmental Engineering. 40, 126–134.

[15] Choi, H.-J.; Choi, Y.; Rhee, S.-W. Estimation on Migration Characteristics of Leachate Using Analysis of Hydraulic Conductivity at Bioreactor Landfill. Waste Manag Res 2020, 38 (1), 59–68.
[16] Venkatesh Reddy, C., Shekhar Rao, D., Kalamdhad, A.S. (2020) Statistical Modelling and Assessment of Landfill Leachate Emission from Fresh Municipal Solid Waste: A Laboratory-Scale Anaerobic Landfill Simulation Reactor Study. Waste. Manag. Res. 38(10), 1161–1175.
[17] Degois, J., Simon, X., Clerc, F., Bontemps, C., Leblond, P., Duquenne, P. (2021) One-Year Follow-up of Microbial Diversity in Bioaerosols Emitted in a Waste Sorting Plant in France. Waste Management. *120*, 257–268.
[18] Gao, X., Xu, Z., Li, Y., Zhang, L., Li, G., Nghiem, L.D., Luo, W. (2021) Bacterial Dynamics for Gaseous Emission and Humification in Bio-Augmented Composting of Kitchen Waste. Science of the Total Environment. 801, 149640.
[19] Chen, Q., Liu, B., Wang, J., Che, J., Liu, G., Guan, X. (2017) Diversity and Dynamics of the Bacterial Community Involved in Pig Manure Biodegradation in a Microbial Fermentation Bed System. *A*nn. Microbiol. 67 (7), 491–500.
[20] Schleifer, K.H., Ludwig, W. (1995) Phylogeny of the Genus Lactobacillus and Related Genera. Systematic and Applied Microbiology.18 (4), 461–467.
[21] Zhao, Y., Wei, W., Tang, L., Wang, D., Wang, Y., Wu, Z., Zhang, W. (2021) Characterization of Aroma and Bacteria Profiles of Sichuan Industrial Paocai by HS-SPME-GC-O-MS and 16S RRNA Amplicon Sequencing. Food Research International. 149, 110667.
[22] Messens, W., Verluyten, J., Leroy, F., De Vuyst, L. (2003) Modelling Growth and Bacteriocin Production by Lactobacillus Curvatus LTH 1174 in Response to Temperature and PH Values Used for European Sausage Fermentation Processes. International Journal of Food Microbiology. 81(1), 41–52.
[23] Liu, Z., Peng, Z., Huang, T., Xiao, Y., Li, J., Xie, M., Xiong, T. (2019) Comparison of Bacterial Diversity in Traditionally Homemade Paocai and Chinese Spicy Cabbage. Food Microbiology. 83, 141–149.
[24] Kim, E., Yang, S.M., Kim, H.Y. (2023) Weissella and the Two Janus Faces of the Genus. Appl. Microbiol. Biotechnol. 107(4), 1119–1127.
[25] Pal, A., Ramana, K.V. (2010) Purification and Characterization of Bacteriocin from Weissella Paramesenteroides Dfr-8, an Isolate from Cucumber (Cucumis Sativus). Journal of Food Biochemistry. 34(5), 932–948.
[26] Uriot, O., Denis, S., Junjua, M., Roussel, Y., Dary-Mourot, A., Blanquet-Diot, S. (2017) Streptococcus Thermophilus: From Yogurt Starter to a New Promising Probiotic Candidate? Journal of Functional Foods. 37, 74–89.
[27] Niemelä, S.I., Oivanen, P. (1992) Transition from Growth to Death in Populations of Klebsiella Terrigena and Other Enterobacteriaceae in Relation to Temperature. FEMS Microbiology Letters. 91(2), 181–185.
[28] Desta, D.T., Kelikay, G.N., Zekwos, M., Eshete, M., Reda, H.H., Alemayehu, F.R., Zula, A.T. (2021) Influence of Fermentation Time on Proximate Composition and Microbial Loads of Enset, (Ensete Ventricosum), Sampled from Two Different Agroecological Districts. Food Sci. Nutr. 9(10), 5641–5647.
[29] Fessard, A., Remize, F. (2019) Genetic and Technological Characterization of Lactic Acid Bacteria Isolated from Tropically Grown Fruits and Vegetables. International Journal of Food Microbiology. 301, 61–72.
[30] Ko, J.A., Kim, W.Y., Park, H.J. (2012) Effects of Microencapsulated Allyl Isothiocyanate (AITC) on the Extension of the Shelf-Life of Kimchi. International Journal of Food Microbiology. 153(1), 92–98.
[31] Choi, E., Kim, J., Choi, I., Ahn, H., Dong, J.I., Kim, H. (2015) Microbial Additives in Controlling Odors from Stored Swine Slurry. Water Air Soil Pollut. 226(4), 104.
[32] Chen, Y.C., Higgins, M.J., Beightol, S.M., Murthy, S.N., Toffey, W.E. (2011) Anaerobically Digested Biosolids Odor Generation and Pathogen Indicator Regrowth after Dewatering. Water Research. 45(8), 2616–2626.

**Received: 29.08.2023**
**Accepted: 13.09.2023**

**CORRESPONDING AUTHOR**

**Chuan Huang**
College of Environment and Ecology
Chongqing University
Chongqing 400044 – China

e-mail: hclsjb@163.com

- © by PSP Volume 32– No. 09/2023 pages 3060-3068 Fresenius Environmental Bulletin

# BIOLOGICAL INHIBITION OF SULFIDE TO IMPROVE POLYMER VISCOSITY INSTEAD OF FORMALDEHYDE DISINFECTANT

**Du Chun'an***

College of Chemical Engineering, Shandong Institute of Petroleum and Chemical Technology, Dongying, 262500, China

## ABSTRACT

Sulfate-reducing bacteria (SRB) are ubiquitous in oilfield wastewater systems. The sulfur ions produced by SRB metabolism lead to viscosity loss of polymer solution during dilution and polymer solution preparation of sewage. To avoid the disadvantages of poor stability and short effective distance in using formaldehyde to sterilize and stabilize viscosity, biological competition method was adopted. Denitrifying inhibitors and functional bacteria were added. Viscosity stability of polymer solution prepared with wastewater after biological treatment were evaluated in the lab. The mechanism of viscosity stabilization was analyzed. The results of lab experiments showed that 30 days after biological treatment, the viscosity of the polymer solution prepared with wastewater was still above 34 mPa.s, and the retention rate of the viscosity was more than 80%, which was similar to that of the polymer solution after sterilization. It could be seen in the analysis of microbial community structure that biological treatment could restrain the activity of SRB and prevent the degradation of polymer by sulfur ion produced by SRB through activating denitrifying bacteria, which could compete with SRB for nutrient substrates. Biological inhibition of sulfur could completely replace bactericidal method in viscosity stabilization of polymer solution .

**KEYWORDS:**
Sulfate-reducing bacteria (SRB), sulfur ions, biological competition method, formaldehyde, polymer, stabilize viscosity

## INTRODUCTION

Oil is the lifeblood of a nation's economic development, and the increasing demand for crude oil poses a serious threat to national energy security. Therefore, improving crude oil recovery is the primary task for solving China's energy crisis. At present, most of China's oil fields have entered the middle and late stages of water injection development, and chemical drive has become an important means to increase and stabilize production in oil fields. The evaluation of China's petroleum resources potential shows that the enhanced oil recovery (EOR) method may increase crude oil recoverable reserves by 1.18 billion tons. Among these, composite drive methods, including polymer flooding, alkali/polymer binary composite flooding, and alkali/surfactant/polymer ternary chemical flooding cover 76% of the reserves[1] . Polymer improves recovery by increasing the viscosity and retention time of the injected fluid, reducing the formation permeability to adjust the suction profile, and improving the hydraulic flow ratio and the actual ripple coefficient. Therefore, polymer solution viscosity is an important factor affecting the effectiveness of chemical drives. Recent studies have shown that, in addition to polymer molecular structure, molecular weight size, hydrolysis degree, and mechanical shear, high valence ions in the effluent, temperature, and other factors affecting polymer viscosity and sulfides in the effluent system can also cause chemical degradation of the polymer. This could, in turn, lead to severe polymer viscosity loss and influence the effect of poly-drive development[2, 3].

At present, the methods used in the oilfield to eliminate the effect of sulfide on the viscosity of the allotment mainly include the application of biocides to kill sulfate-reducing bacteria (SRB), aeration, and oxygen-based removal of sulfur[4-6]. However, the former involves high consumption of biocides in the effluent containing aggregates and offers poor results due to the development of bacterial resistance[7]. Meanwhile, aeration and sulfur removal may lead to a polymer solution with lower viscosity at the wellhead compared with the expected value. The evaluation and control of the degree of aeration in practical application highlight the generalization. At the same time, neither of these methods is effective in killing the bacteria in biofilms on the wall of the injection pipeline and the inner wall of the wellbore. This leads to poor results in the pipeline transmission from the aggregation station to the injection station and during injection into the aggregation well; polymer solution viscosity is lost due to the metabolic production of sulfide from SRB that grows along the route[8]. Viscosity loss at the injection station and the single well pipeline accounts for approximately 77% of the total viscosity loss of the polymer solution, and the viscosity of the polymer

solution was found to be only 17 mPa.s at some wellheads and less than 10 mPa.s at 1,000 m downhole within the injection well.

Following the tertiary oil recovery conducted at Gudong Oil Production Plant, to ensure the stability of the polymer solution viscosity, formaldehyde was continuously injected. However, long-term human contact with formaldehyde may cause cancer. In addition, the formaldehyde solution has a self-polymerizing effect, and during injection, it can very easily cause blockage in the formaldehyde tank, level meter, and pipeline, seriously affecting its normal use. Therefore, there is an urgent need to replace formaldehyde injection to avoid dilution of sewage and ensure stable viscosity of the polymer solution[9].

In this paper, we compared the viscosity stabilization effect of the biological method and formaldehyde injection method on the viscosity of polymer solution. Then we revealed the mechanism of SRB inhibition by the biological method to improve the viscosity of polymer solution by analyzing the changes in community structure, polymer microscopic morphology, and molecular weight during the inhibition process. The findings of this study will provide theoretical guidance for the development of sulfur inhibition and sulfur removal by the biological method to improve the viscosity of polymer solution.

## MATERIALS AND METHODS

**Experimental materials and instruments.** Polyacrylamide (HPAM) :The HPAM was purchased from Shandong Polymer Biochemicals Co., Ltd., hydrolysis degree 23%, solid content 90%.

Main instruments: Brookfield DV-Ⅲ viscometer (Brookfield, USA); S-4800 Cold field scanning electron microscope (Hitachi); Gel chromatograph (Wyatt, USA); Optilab-rex Differential refractive Index Detector (Wyatt, USA); DAWN HELEOS eighteen Angle Laser Light Scatterer (Wyatt, USA).

**Experimental methords. 1.2.1 Measurement of the HPAM solution viscosity.** Distilled water was used to prepare 5000 mg/L polymer mother liquor, followed by dilution to 1700 mg/L with untreated sewage, formaldehyde sterilized sewage and biological sewage, respectively. The solutions were then incubated hermetically at 60℃, and sampled regularly to test the viscosity. The viscosity of the HPAM solution was measured by Brookfield DV-III viscometer. The No. 0 rotor was selected at a speed of 6 rpm and a temperature of 70 °C.

**Quantification of sulfide ions.** Methylene blue spectrophotometry (GB/T 16489) was used for the determination of the sulfide ion concentration.

**Microscopic morphology analysis.** Distilled water was used to prepare 5000 mg/L polymer mother liquor, followed by dilution to 1700 mg/L with untreated sewage, formaldehyde sterilized sewage and biological sewage, respectively. After being incubated hermetically for 10d, a scanning electron microscope was used to observe their microscopic morphologies with an accelerating voltage of 5 kV and magnification of ×20.0 K.

**Measurement of the molecular weight.** For the solutions in 2.3, the molecular weight distribution was measured by gel chromatography refractive index detector. The refractive index was 1.331. The weight average molecular weight ($M_W$) was directly determined by a light scattering instrument with a laser of 658.0 nm.

## RESULTS

**Viscosity stability evaluation.** The viscosity test results are shown in Figure 1. The results show that the viscosity of diluted sewage after 30 d of biological sulfur suppression treatment [3–6] and sterilization treatment still reaches more than 34 MPa.s, and the viscosity retention rate exceeds 80%. Meanwhile, the viscosity stability of untreated sewage is poor, and the viscosity retention rate is only 22%. This shows that biological sulfur suppression treatment can be used to replace bactericidal treatment for dilution and polymerization and ensures viscosity stability of the polymer solution.

**Biological method of sulfur suppression and viscosity stabilization mechanism.** To clarify the mechanism of viscosity stabilization of dilution with treated sewage using different methods, the microscopic morphology and molecular weight changes of the polymer and the structural changes of the bacterial community were analyzed.

**Polymer micromorphology.** The microscopic morphology of polymer solutions prepared with untreated, formaldehyde, and biogenic-sulfur-suppression-treated effluent after 10 d of were observed. The polymer formulated with untreated effluent had a loose spatial lattice structure with uneven distribution, which implied that the polymer had been degraded and the structure had been damaged. In contrast, the polymer formulated with biogenic sulfur suppression treated effluent exhibited a superior spatial structure and good homogeneity. The electron micrographs are shown in Figure 2.

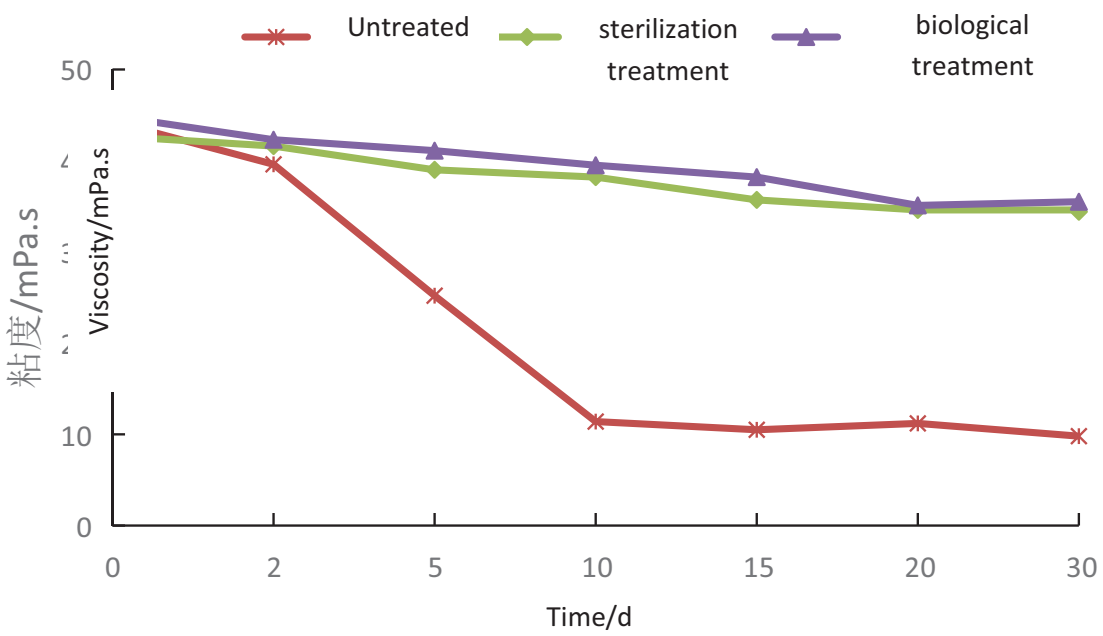
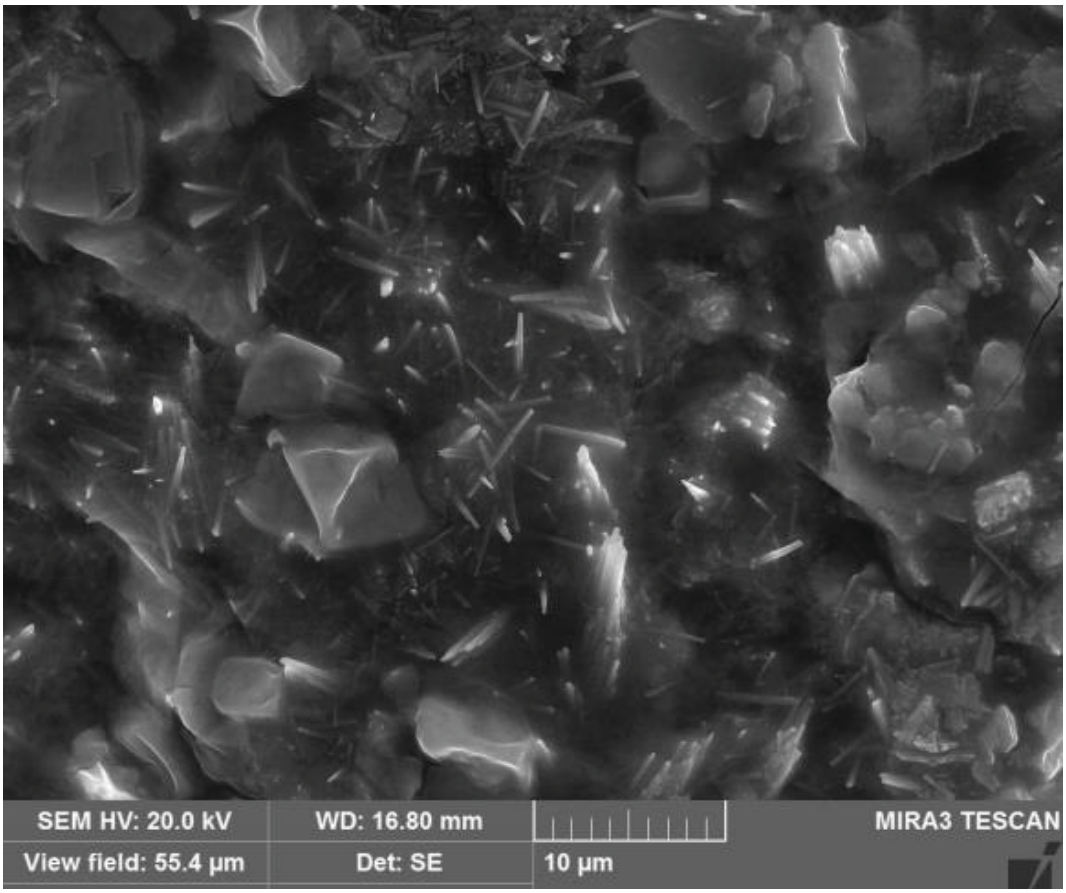
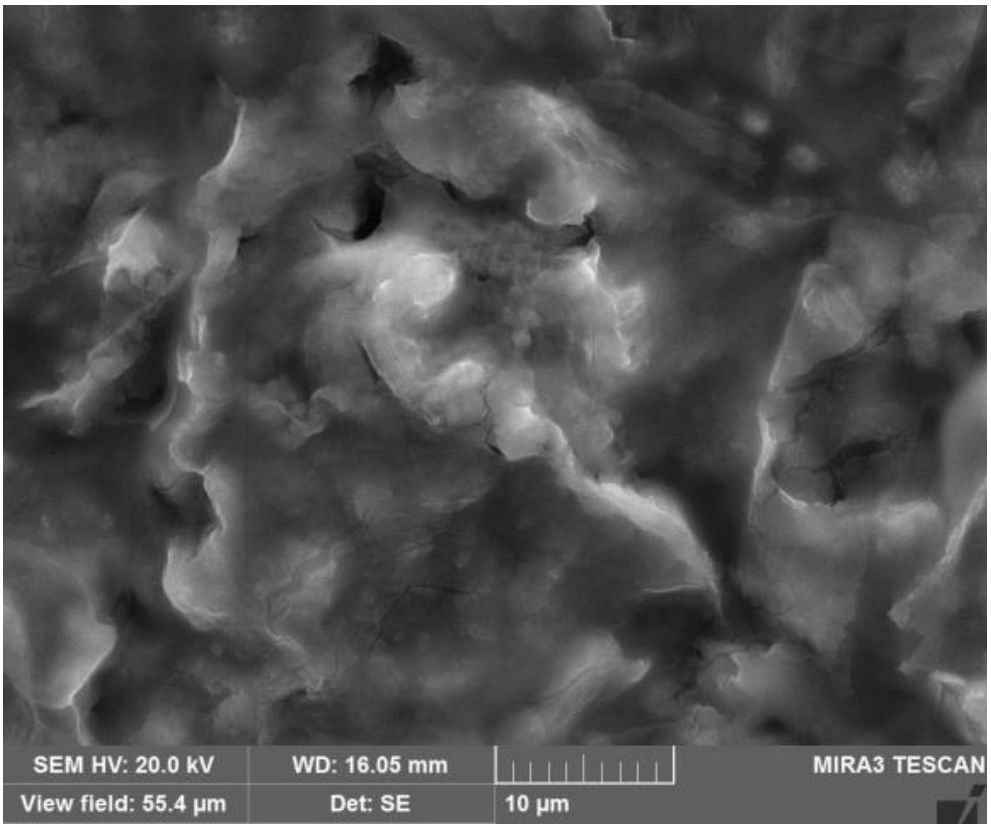


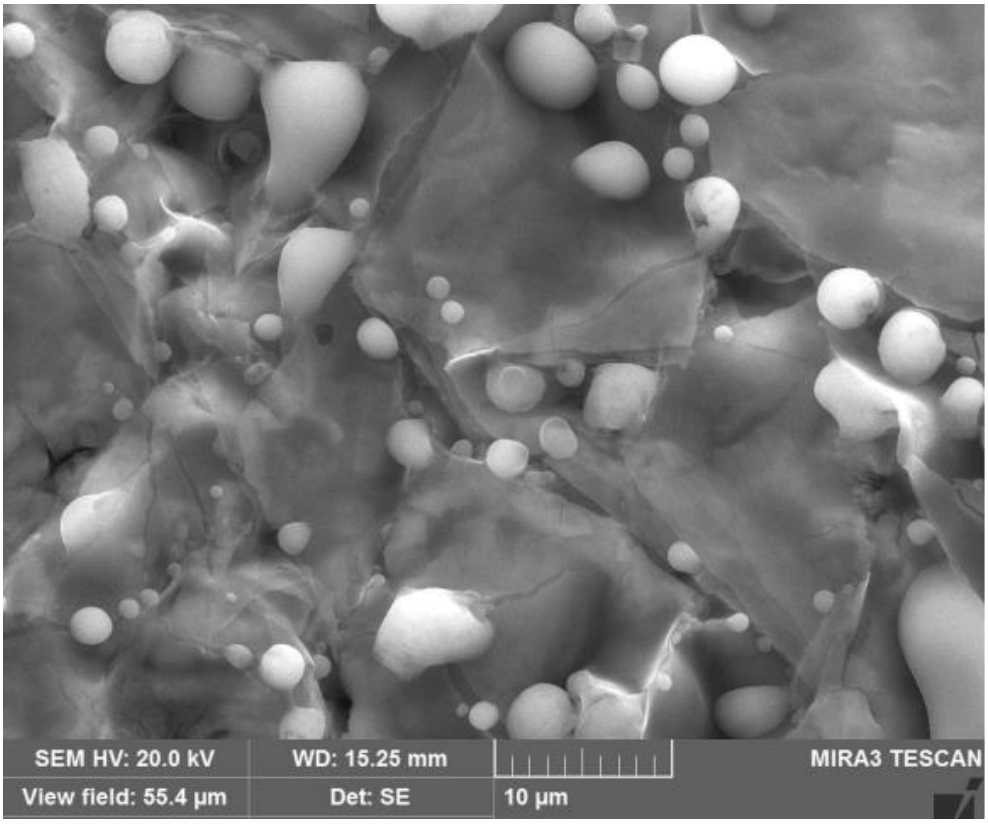

**FIGURE 1**
**Viscosity stability of dilution with treated sewage using different methods**




**a MY0**




**b MYPA**




**c MYBC**

**FIGURE 2**

**Microscopic morphology of polymer solutions prepared with different water**

**(MY0- polymer solutions prepared with untreated water, MYPA- polymer solutions prepared with water treated by formaldehyde, MYBC- polymer solutions prepared with biogenic-sulfur-suppression-treated water)**

**TABLE 1**
**Change of molecular weight**

| | MY0 | MYPA | MYBC |
|---|---|---|---|
| molecular weight distribution(Mw/Mn) | 1.108 | 1.029 | 1.016 |
| molecular weight (Mn) /g/moL | $1.056\times10^6$ | $3.486\times10^6$ | $3.512\times10^6$ |
| radius of gyration(Rz) /nm | 87.4 | 193.2 | 199.5 |

**TABLE 2**
**The variation of SRB concentration**

| time | MY0 | | MYPA | | MYBC | |
|---|---|---|---|---|---|---|
| | SRB | $S^{2-}$ | SRB | $S^{2-}$ | SRB | $S^{2-}$ |
| 0d | 600 | 0.6 | 0 | 0 | 600 | 0 |
| 6d | 2500 | 3.5 | 0 | 0 | 2.5 | 0 |
| 10d | 250 | 4.1 | 0 | 0 | 0 | 0 |

**Polymer molecular weight variation.** The molecular weights of the 10 d samples of untreated sewage, biogenic sulfur suppression, and post-sterilization sewage diluted with polymerization solvent were tested, and the results are shown in Table 1. It can be seen that the molecular weight distribution of the polymer of the untreated sewage diluted with polymerization solution was quite broad, with the average molecular weight dropping to $1.056\times10^6$ g/mol and the radius of gyration being 87.4 nm, while the respective molecular weights of the samples of biogenic sulfur suppression and post-sterilization sewage diluted with polymerization solvent were 199.5 and 191.7 nm. The respective molecular weights of the polymers were $3.512\times10^6$ g/moL and $3.486\times10^6$ g/moL, with respective radii of gyration of 199.5 and 191.7 nm. It was inferred that the SRB metabolism in the untreated effluent produced sulfides that caused polymer chain breakage and degradation to produce small molecules. In addition, the polymer degradation was inhibited by both biological sulfur inhibition treatment and bactericidal treatment.

**Changes in SRB Concentration.** The variation in SRB concentration in different samples by the MPN method showed that the SRB in the untreated effluent reached 600/mL, which then increased to 2500/mL after 6 d of dilution and aggregation, indicating the proliferation of SRB (table 2). The SRB was undetectable after 10 d of dilution and aggregation of the biocidal treated effluent and decreased to 2.5 SRB/mL after 6 d of dilution and aggregation of the biogenic sulfur suppression treated effluent. The $S^{2-}$ produced by the sulfate-reducing bacteria caused the polymer chains to break, resulting in a loss of viscosity. The bactericides inhibited the degradation of the polymer by directly killing the SRB and inhibiting sulfide production. The biological sulfur inhibition treatment resulted in the inhibition of SRB activity; no sulfide production was observed during the experiment, and the polymer viscosity was stable.

**Changes in microbial community structure during biogenic sulfur suppression. 1) Bacterial community diversity analysis.** The number and diversity index of each water sample OUT for the different treatment methods are shown in the table 3. The coverage of each sample library reached over 99.7%, indicating that this sequencing can reflect the real composition of the bacterial community. The number of samples OUT decreased for both the resting initial polymer solution for 10 d and the biological method treatment for 10 d, while the number of samples OUT increased for the biocide dosed samples. The Chao abundance index showed that the bacterial community abundance increased for the resting initial mother solution and biocide dosed systems and decreased for the biological method inhibition system. The Shannon diversity index showed an increase in bacterial diversity for all three modalities, with the highest Shannon index and lowest Simpson index for the bioprocess inhibition system, indicating that biocompetitive inhibition favors the growth of a wide range of microorganisms.

**TABLE 3**
**The number and diversity index of different samples**

| | MY | MY0 | MYPA | MYBC |
|---|---|---|---|---|
| Tag number | 30791 | 32265 | 33851 | 32743 |
| Coverage | 0.9963 | 0.9976 | 0.9967 | 0.9979 |
| OTUs | 452 | 345 | 569 | 378 |
| Chao | 537.28 | 608.72 | 624.62 | 507.04 |
| Shannon diversity | 3.01 | 3.16 | 3.21 | 3.26 |
| Simpson | 0.098 | 0.088 | 0.094 | 0.070 |

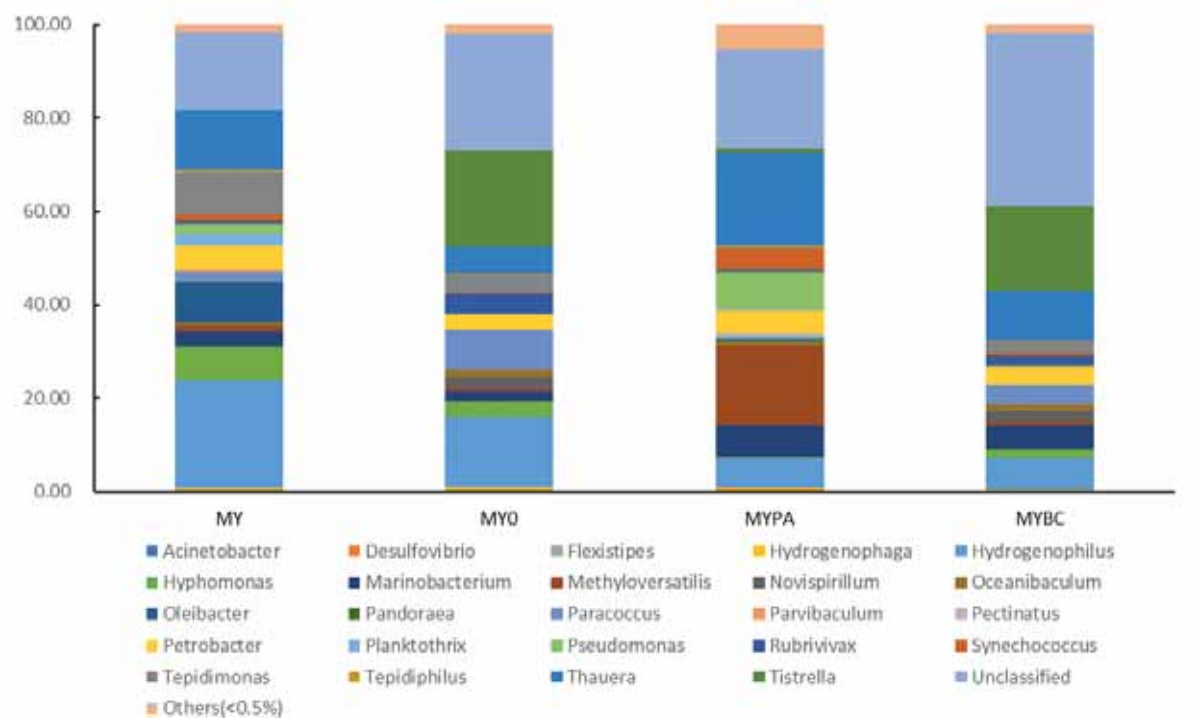



**FIGURE 3**
**Results of high-throughput sequencing**

**2) Changes in the composition of the bacterial community.** The results of high-throughput sequencing of bacteria for each sample are shown in Figure 3.

As seen in the figure, the abundance of bacteria with nitrate reduction function, such as *Marinobacterium*, *Thauera*, and *Hyphomonas*, decreased after 10 d polymer solution addition. In the absence of an activator, this group of bacteria was not dominant in the biological competition process. Finally, the abundance of *Paracoccus*, *Rubrivivax*, *Tistrella*, and other genera with petroleum hydrocarbon degradation functions increased significantly.

The abundance of formaldehyde degrading bacteria such as *Pseudomonas* and *Methyloversatilis* after formaldehyde polymer solution treatment increased from 2.26% and 1.20% to 7.97% and 17.08% in the polymer solution, respectively.

The abundance of bacteria such as *Marinobacterium* and *Thauera* with nitrate-reducing functions increased in the polymer solution treated by the biological inhibition method. These genera can use nitrate or molecular oxygen as electron acceptors to oxidize the reduced sulfide and obtain the energy required for growth and reproduction. An increased abundance of *Oceanibaculum*, a bacterium genus in the Indian Ocean capable of reducing nitrate to nitrite but incapable of denitrification, was also detected. In addition, the relative abundance of *Petrobacter* and *Hydrogenophilus* decreased from 5.06% and 22.9% to 3.87% and 6.93%, respectively. *Petrobacter* is an important genus of oil recovery bacteria with surfactant-producing properties and they degrade petroleum hydrocarbons. The reduced abundance of this bacterium indicates that the addition of high inhibitor concentrations activated the denitrifying bacteria in the effluent, which inhibited the activity of *Petrobacter* while competing with SRB for carbon sources. The altered ecology resulted in a complex competitive inhibition relationship in the system. *Hydrogenophilus* is a hydrogen-oxidizing bacterium that can use $H_2$ as an energy source and can oxidize sulfur for energy. The decrease in abundance of this bacterium is mainly due to the addition of inhibitors that remove sulfide, the energy source of *Hydrogenophilus*, from the system.

The presence of about 20% of unclassified bacteria in each sample was also observed, thus indicating a complex distribution of species within the oilfield wastewater system and the possible presence of new, unculturable genera.

**(3) Changes in the composition of archaeal communities.** The mutualistic interaction between archaea and bacteria can achieve synergistic degradation of refractory organic matter. In the experiment, a total of nine dominant bacteria (>0.5% abundance), including *Methanomethylovorans*, *Methanosaeta*, *Methanothermobacter*, and *Methanoculleus* were detected (Figure. 4). The relative abundance of hydrogen-utilizing methanogenic genera such as *Methanoculleus* and *Methanolinea* decreased in the untreated-effluent-formulated polymer solution, while that of *Methanomethylovorans* (methyl-utilizing methanogenic bacteria) and *Methanosaeta* (acetic acid trophic methanogenic bacteria) increased.

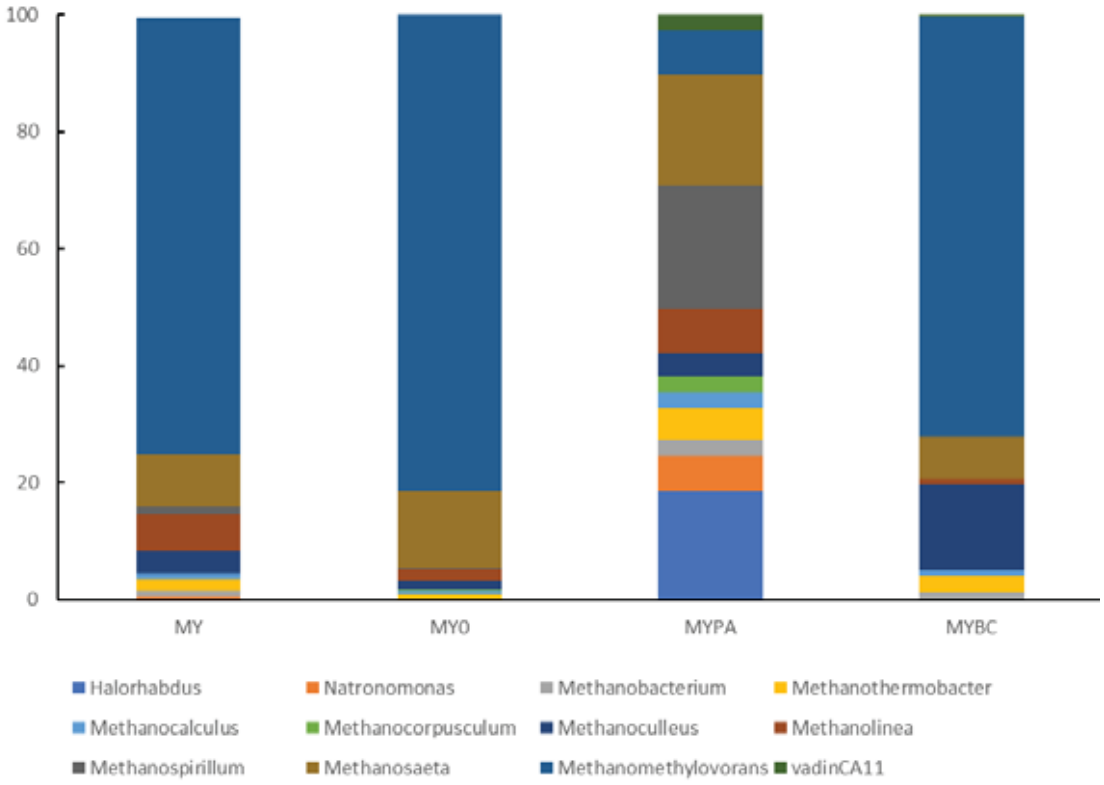



**FIGURE 4**
**Changes in the composition of archaeal communities**

This was because the polymer solution prepared from the untreated effluent underwent degradation of the polymerization solvent, thus generating low molecular weight nutrient substrates that can be utilized by this group of archaea. The relative abundance of *Methanoculleus* and *Methanothermobacter*, which may be important functional bacteria in the denitrification process, increased in the polymer solution after biological treatment.

## DISCUSSION

There are two main reasons for the reduction of polyacrylamide (HPAM) solution viscosity caused by SRB. One is that SRB directly uses polyacrylamide as a carbon or nitrogen source for growth and reproduction. The polymer is then degraded to small molecules, causing viscosity reduction [10-13]. The second reason is that $S^{2-}$ produced by SRB metabolism and $Fe^{2+}$ produced by corrosion with SRB cause molecular chain breakage, and the degradation products can be used by SRB as a nutrient substrate, thus promoting the growth and reproduction of SRB to further degrade the polymer [14]. The reduction of polymer viscosity caused by SRB in oilfield wastewater systems is dominated by the latter. Therefore, the key to inhibiting the loss of polymer solution viscosity is to inhibit SRB activity, so that it cannot produce $S^{2-}$.

No SRB was detected and no $S^{2-}$ was generated in the experimental system dosed with formaldehyde. Formaldehyde acts on bacterial proteins (including enzymes) to alkylate them, causing protein denaturation and coagulation, resulting in microbial death. Although some bacteria such as *Pseudomonas* and *Methyloversatilis* could survive and proliferate, the polymer viscosity was found stable during the experiments, because there was no $S^{2-}$ causing polymer chain breakage, and these bacteria had difficulty using the polymer as a nutrient substrate.

In the biological treatment process, by adding activators to the SRB environment, first the $SO_4^{2-}$ in the electron transfer process is reduced, then the dormant NRB is activated, and growth and reproduction are accelerated. Then, the NRB competes with the SRB for living space and nutrient substrate (Figure. 5). The preferential use of nutrient substrate by NRB leads to nutrient insufficiency for SRB, thus limiting SRB activity. Unlike the biocide injection treatment method, the experimental process of biological treatment did not produce $S^{2-}$, although the presence of SRB was observed. In addition, the polymer viscosity remained stable for a long time, which was due to the preferential use of nutrient substrate by the activated DNB, due to which the already scarce bioavailable nutrients in the oilfield effluent were depleted, and the SRB became inactive and consequently died due to the lack of living space.

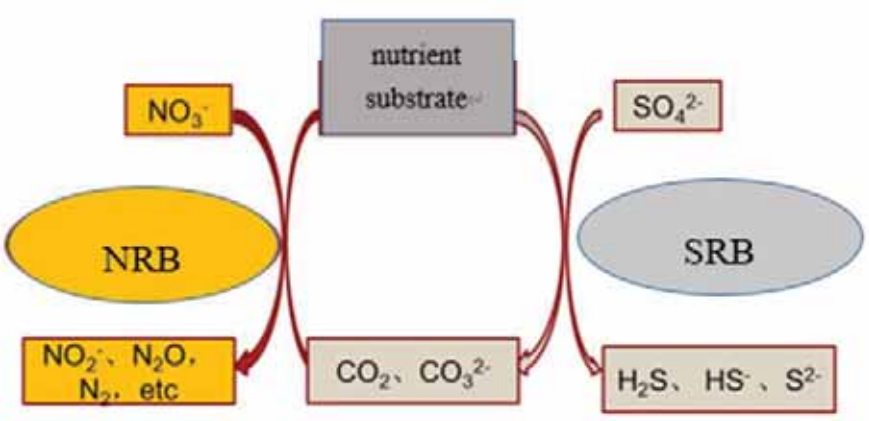



**FIGURE 5**
**Inhibiting mechanism**

In addition, the presence of $NO_2^-$ was monitored during the experiments. $NO_2^-$ can inhibit sulfite reductase activity, in turn preventing the catalytic reduction of sulfite to sulfide.

The abundance of other bacteria such as *Petrobacter* and *Hydrogenophilus* also changed significantly during the experiment. This was because the oilfield wastewater system is a complex "bioreactor," containing a large number of microbial populations. In addition to NRB, SRB, and sulfur-oxidizing bacteria, there are also iron bacteria, petroleum hydrocarbon degradation bacteria, methanogenic archaea, etc. These bacteria will utilize carbon sources for growth and reproduction and are influenced by various factors such as metabolic pathways, environmental factors, biological enzymes, substrate transfer, and so on. The interaction between each group of bacteria and their ability to compete for nutrient substrates is complex, with abundant reaction kinetics. It is necessary to further study the metabolic capacity and competitive cooperation at the community and individual levels with the aid of quantitative gene, next-generation sequencing, and macro-omics technologies. The interrelationships affecting degradation performance can be studied with the aid of multi-dimensional macro-omics and environment-dependent experiments.

## CONCLUSIONS

1) The viscosity of the diluted and aggregated effluent after biological sulfur suppression treatment still reaches more than 34 mPa/s after 30 d. The viscosity retention rate exceeds 80%, which is comparable to the viscosity of the aggregated effluent after sterilization treatment.

2) Biological sulfur suppression mainly occurs through activation of the denitrifying bacteria in the effluent to compete with SRB for nutrient substrates, thereby limiting SRB activity and preventing degradation of polymers by sulfur ions produced by SRB metabolism.

## REFERENCES

[1] Liao, G.Z., Wang, Q., Wang, H.Z., Liu, W.D., Wang, Z.M. (2017) Chemical flooding developmenr status and prospect. Acta Petrolei Sinica.38, 196-207.

[2] Liu, J.F., Feng, J.Y., Hu, H., Li, C.Y., Yang, S.Z., Gu, J.D., Mu, B.Z. (2018) Decrease in viscosity of partially hydrolyzed polyacrylamide solution caused by the interaction between sulfide ion and amide group. Journal of Petroleum Science and Engineering. 170, 738-743.

[3] Piceno, Y.M., Reid, F.C., Tom, L.M., Conrad, A.E., Bill, M., Hubbard, C.G., Fouke, B.W., Graff, C.J., Han, J., Stringfellow, W.T., Hanlon, J.S., Hu, P., Hazen, T.C., Andersen, G.L. (2014) Temperature and injection water source influence microbial community structure in four Alaskan North Slope hydrocarbon reservoirs. Front.Microbiol. 5, 13.

[4] Zhang, Z.Y., Ni, M.N., Tian, L., Qin, Y.C., Zhuang, D., Cheng, Y.H., Lin, Y. (2021) Competition and cooperation of sulfate reducing bacteria and five other bacteria during oil production. Journal of Petroleum Science and Engineering. 203, Pp. 108688.

[5] Wang, J.L. Hou, B.S., Xiang, J., Chen, X.D., Gu, T.Y., Liu, H.F. (2019) The performance and mechanism of bifunctional biocide sodium pyrithione against sulfate reducing bacteria in X80 carbon steel corrosion. Corrosion Science. 150, 296-308.

[6] Lai, R.Q., Li, Q., Shen, H. Liu, S.Q., Luo, Y.J., Zhang, Z.Z., Sun, S.S. (2020) Bio-competitive exclusion of sulfate-reducing bacteria and its anticorrosion property. Journal of Petroleum Science and Engineering. 194, Pp. 107480.

[7] Johnson, R.J., Folwell, B.D., Wirekoh A., , Frenzel, M., Skovhus, T.L. (2017) Reservoir Souring – Latest developments for application and mitigation. Journal of Biotechnology. 256, 57-67.

[8] Veshareh, M.J., Kjeldsen, K.U., Findlay, A.J., Nick, H.M., Røy, H., Marietou, A. (2021) Nitrite is a more efficient inhibitor of microbial sulfate reduction in oil reservoirs compared to nitrate and perchlorate: A laboratory and field-scale simulation study. International Biodeterioration & Biodegradation. 157, Pp. 105154.
[9] Eroini, V., Anfindsen, H., Mitchell, A.F. (2015) Investigation, classification and remediation of amorphous deposits in oilfield systems. In: Paper SPE-173719. SPE International Symposium on Oilfield Chemistry. Society of Petroleum Engineers, Houston, USA. 1-5.
[10] Xue, Y., Voordouw, G. (2015) Control of Microbial Sulfide Production with Biocides and Nitrate in Oil Reservoir Simulating Bioreactors. Frontiers in Microbiology. 6, 1387.
[11] Wijdan, H., Kawther, H. (2017) Inhibition of biogenic hydrogen sulfide produce by Sulfate Reducing Bacteria isolated from oil fields in Basra by nitrate based treatment. Journal of Petroleum Research & Studies. 15, 88-106.
[12] Zhou, X., Liu, L., Chen, C., Ren, N.Q., Wang, A.J., Lee, D.J. (2011) Reduction of produced elementary sulfur in denitrifying sulfide removal process. Appl. Microbiol. Biotechnol. 90, 1129-36.
[13] Kamarisima, Hidaka, K., Miyanaga, K., Tanji, Y. (2018) The presence of nitrate- and sulfate-reducing bacteria contributes to ineffectiveness souring control by nitrate injection. International Biodeterioration & Biodegradation. 129, 81-88.
[14] Agrawal, A., Park, H.S., Nathoo, S., Gieg, L.M., Jack, T.R., Miner, K., Ertmoed, R., Benko, A., Voordouw, G. (2012) Toluene depletion in produced oil contributes to souring control in a field subjected to nitrate injection. Environ. Sci. Technol. 46, 1285-1292.

**Received:** **28.08.2023**
**Accepted:** **09.09.2023**

## CORRESPONDING AUTHOR

**Du Chun'an**
College of Chemical Engineering,
Shandong Institute of Petroleum and Chemical Technology
Dongying 262500 – China

e-mail: 61522463@qq.com

## AUTHOR INDEX

## SUBJECT INDEX

# FEB – GUIDE FOR AUTHORS

**General**

FEB accepts original papers, review articles, short communications, research abstracts from the entire sphere of environmental-chemistry,-biology,-microbiology,- technology, -biotechnology and-management, furthermore, about residue analysis/ and ecotoxicology of contaminants.

Acceptance or no acceptance of a contribution will be decided, as in the case of other scientific journals, by a board of reviewers. Papers are processed with the understanding that they have not been published before (except in form of an abstract or as a part of a published lecture, review or thesis);that they are not under consideration for publication elsewhere; that their publication has been approved by all co-authors, if any, as well as- tacitly or explicitly- by the responsible authorities at the institute where the work has been carried out and that, if accepted, it will not be published elsewhere in the same form, in either the same or another language, without the consent of the copyright holders.

**Language**

Papers must be written in English. Spelling may either follow American (Webster) or British (Oxford) usage but must be consistent. Authors who are less familiar with the English language should seek assistance from proficient colleagues in order to produce manuscripts that are grammatically and linguistically correct.

**Size of manuscript**

Review articles should not exceed 30 typewritten pages. In addition up to 5 figures may be included.

Original papers must not exceed 14 typewritten pages. In addition up to 5 figures may be included.

Short-Communications should be limited to 4 typewritten pages plus not more than 1 illustration.

Short descriptions of the authors, presentation of their groups and their research activities (with photo) should together not exceed I typewritten page. Short research abstracts should report in a few brief sentences (one-fourth to one page) particularly significant findings. Short articles by relative newcomers to the chemical innovation arena highlight the key elements of their Master and PhD-works in about I page.

Book Reviews are normally written in-house, but suggestions for books to review are welcome.

**Preparation of manuscript**

Dear authors,

FEB is available both as printed journal and as online journal on the web. You can now e-mail your manuscripts with an attached file. Save both time and money. To avoid any problems handling your text please follow the instructions given below:

When preparing your manuscripts have the formula K/SS (Keep It Simple and Stupid) in mind. Most word processing programs such as MS-Word offer a lot of features. Some of them can do serious harm to our layout. So please do not insert hyperlinks and/or automatic cross-references, tables of contents, references, footnotes, etc.

1. Please use the standard format features of your word processor (such as standard.dot for MS Word).
2. Please do not insert automatisms or secret link-ups between your text and your figures or tables. These features will drive our graphic department sometimes mad.
3. Please only use two fonts for text or tables "Times New Roman" and for graphical presentations "Arial".
4. Stylesheets, text, tables and graphics in shade of grey
5. Turn on the automatic language detection in English (American or British)
6. Please - check your files for viruses before you send them to us!!

**Manuscripts should be uploaded on our website prt-parlar.de**

Thank you very much!

## STRUCTURE OF THE MANUSCRIPT

**Title page:** The first page of the manuscript should contain the following items in the sequence given: A concise title of the paper (no abbreviations). The names of all authors with at least one first name spelled out for every author. The names of Universities with Faculty, City and Country of all authors.

**Abstracts:** The second page of the manuscript should start with an abstract that summarizes briefly the contents of the paper (except short communications). Its length should not exceed 150-200 words. The abstract should be as informative as possible. An extended repetition of the paper's title is not considered to be an abstract.

**Keywords:** Below the Summary up to 6 key words have to be provided which will assist indexers in cross-indexing your article.

**Introduction:** This should define the problem and, if possible, the frame of existing knowledge. Please ensure that people not working in that particular field will be able to understand the intention. The word length of the introduction should be 150 to 300 words.

**Materials and methods:**
Please be as precise as possible to enable other scientists to repeat the work.

**Results:** Only material pertinent to the subject must be included. Data must not be repeated in figures and tables.

**Acknowledgements:** Acknowledgements of financial support, advice or other kind of assistance should be given at the end of the text under the heading "Acknowledgements". The names of funding organisations should be written in full.

**References:** Responsibility for the accuracy of references rests with the authors. References are to be limited in number to those absolutely necessary. References should appear in numerical order in brackets and in order of their citation in the text. They should be grouped at the end of the paper in numerical order of appearance. Abbreviated titles of periodicals are to be used according to Chemical or Biological Abstracts, but names of lesser known journals should be typed in full. References should be styled and punctuated according to the following examples:

ORIGINAL PAPERS:
l. Author, N.N. and Author, N.N. (Year) Full title of the article. Journal and Volume, first and last page.

BOOK OR PROCEEDING:
2. Surname, N., Surname, N.N., Surname, N.N., Surname, N. (Year) Title of the contribution. In: Title of the book or proceeding. Volume, Publisher, City, first and last page

DOCTORAL THESIS:
3. Author, N.N. (Year) Title of the thesis, University and Faculty, City

UNPUBLISHED WORK:
Papers that are unpublished but have been submitted to a journal may be cited with the journal's name followed by "in press". However, this practice is acceptable only if the author has at least received galley proofs of his paper. In all other cases reference must be made to “unpublished work" or "personal communication".

**Discussion and Conclusion:** This part should interpret the results in reference to the problem outlined in the introduction and of related observations by the author/s or others. Implications for further studies or application may be discussed. A conclusion should be added if results and discussion are combined.

**Corresponding author:** The name of the corresponding author with complete postal address