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APPLICATION OF HACCP SYSTEM AT THE LEVEL OF A CENTRAL UNIVERSITY CATERING IN SAIDA CITY (MICROBIOLOGICAL STUDY)

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ABSTRACT

HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards. Hygiene of food refers to is the measures and conditions necessary to control the dangers (biological, chemical and physical) and to guarantee the specific character of the food.

Human consumption of food taking into account the intended use and also is defined as a set of simple rules to help prevent food poisoning so that food can be consumed without in complete safety. The implementation of this system at the Student Cafeteria (University of Saida), has allowed us to retain some CCPs linked to microbiological risks at the preparation and distribution levels, as well as surfaces, materials, air and personnel. The results show non-compliance with hygiene practices for the premises, materials and equipment. The hygiene and behavior of staff during the preparation and the distribution of food was unsatisfactory. The storage conditions for raw foods were unacceptable. In order to guarantee student safety, we have proposed certain corrective measures to remedy any malfunctions. Employee awareness and training in food quality and safety is essential.

KEYWORDS:
HACCP, Food, CCP, Microbiological risks, meals, Hygiene

INTRODUCTION

The HACCP system is a food hazard analysis and control system that was perfected at the end of the 1960s. The Pillsbury Company (United States) developed it at the request from NASA who wanted to avoid food poisoning for astronauts on mission. Since then, the method has continued to evolve, improve and respond [1], [2]. It was in 1971 that the HACCP concept was presented publicly for the first time by The Pillsbury Company. The completion of the use of the principles of the HACCP system by the Food and Drug Administration in the USA was carried out in 1974, for the elaboration of the health regulations for weakly acidic products. In 1993, the Codex Alimentarius published guidelines for the application of HACCP system. The same year, through Directive 93/43/EC, the European Union made it mandatory to apply the principles of HACCP in the food businesses of its Member States [3], [4].

In 1995 France introduced the HACCP concept of catering. Since the year 2000 the majority of companies (large, medium and small) have adopted the food safety system in Europe [5], [6]. In 2005, the ISO 22000 standard incorporated the HACCP system among its principles [7]. Since 2009, Algerian legislation on food safety has provided that companies must carry out a risk analysis according to the principles of the HACCP of the codex alimentarius [8], [9]. HACCP is the English abbreviation of ≪Hazard Analysis Critical Control Points≫, that is to say “risk analysis and critical control points” is a method used to identify, assess and control hazards that threaten product safety based on scientific and coherent bases [10], [11].

MATERIALS AND METHODS

Presentation of the internship location. We carried out our study in the central university catering headquarter, which was inaugurated in 2014, and meets the needs of 800 students. Daily visits for 3 weeks were carried out to check on site the progress of the various culinary preparation operations.

Description of central university restaurant. The following apply to the restaurant which served only lunch:

Two positive cold rooms (one of which was working): for the storage of yogurt, juice, kosher food and eggs;

Vegetable storage room: for storing potatoes, onions, carrots and tomatoes;

 Food storage room: for the storage of spices, olives, concentrated tomatoes, pulses and oil;

TABLE 1
Central University Catering Checklist

	Headings	Ascertainment
Storage of food items	Cold room	Existing: yes x no Cleaning: yes X no
	Feeding chamber	The food items are placed: Good X bad
	The vegetable room	Cleaned: yes X no
	The butcher's shop	Cleaned: yes X no
	Food Items	Separation Respected: yesX no
	Transportation	Respect of the cold chain: Yes no x Refrigerated car: yes no x
	Follow-up register	Existence :yes no x Recording Regulation: yes no x
	Temperature	Control: yes no x
	The preparation culinury room	Vast: yes x no
Preparation methodology	Preparation	Disinfection :Adequate no x/ Quality of water used: Adequate no x
	Preparation medium	Respect of hygienic conditions: yes no x place of preparation: Table x floor
	D.F and D.P products used	Verification : yes x no
	Method of preparation	Professional: yes no x
	Clothing	Wearing work clothes:The blouse: yes x no/ Headgear yes no x Non-slip shoes: yes no x
		Shaved beards: yes no x hand washed: yes no x nail clipped: yes no For some people x
		Graduate staff: yes no x
	Personal hygiene	Medical monitoring: yes x no Periodicity of the control: every 6 months
	Training	Existing Non-existent x
Service	Local	Aération : Good Average Bad x Presence of pets: yes no x
	Drinking Water	Availability: yes x no
Local	Walls	Cleaned: Certain
	Ceiling	Cleaned: yes no/ Sign of deterioration: yes x no
	Doors and windows	Cleaned: yes no x
	Floor	Cleaned: Good Bad x
	Lighting	Sufficient: yes no x
	Aeration	Sufficient: yes no
	Health facility	Presence of sinks: yes no x Presence of soap dispenser yes no x Notices are posted in appropriate places calling on employees to wash their hands: yes no x Displayed notices of protection against coronavirus: yes no x notices displayed for the bib holder: yes no x
	Drains	Sufficient number: yes no x
Equipment	Trash can	Closed : yes no x
	Animals	Pets: yes no x Flies and mosquitoes yes no x
	Dishes	Cleaning: Good bad
	Thermometer	Presence: yes no x
	butcher's table	Presence of holes: yes x no x
Preparation methodology	Utensils Meat cutter	Arranged: yes no Respect hygiene rules: yes no x

 Two changing rooms (for women and men); Student dining room; kitchen; butchery for washing, chopping, cutting and boning of fresh meat ; Sanitary installation.

Description of catering staff. The restaurant had with a single team which is made up of 1 cook, 7 waiters (3 women and 4 men), 2 cleaners and 2 controllers.

Restoration Inspection. We carried out a control at the level of the restoration (Table 1)

Analysis of the operation of the restoration. A. The kitchen and the distribution area are not cleaned which allows for the spread of bacteria;

B. The kitchen floor is deteriorated;

C. There are water leaks (broken faucets);

D. The butcher's table is poorly cleaned and contains holes that promote the growth of pathogenic bacteria;

E. Food items: there is no storage room for bread;

F. Clothing: staff do not wear caps and gloves during distribution (hair is a source of contamination);

G. Personal hygiene: beards, dirty hands and nails: allow contamination between the raw food and the personnel;

H. The same person may be used for different tasks in different sectors;

I. Ceiling: existence of spiders;

J. Trash can: always open and is right next to dishes (dishes) with an unpleasant smell, no waste management system;

K. Cleaning of dishes: it is done in poor hygienic conditions;

L. The absence of air conditioners for ventilation

Material. The material on which our study focused concerns ready meals intended for students. We chose the first course cooked at lunch.

The cooked dish chosen is composed of:

✓ Lentil soup; Beet salad; Green salad; Chicken; Kosher and Juice.

A. Lentil soup: this soup is made up of: Lentils; Oil; Vegetables: onions, tomatoes, carrots and potatoes; Spices: Salt, Cumin, Black pepper, Red pepper and Concentrated tomato.

B. Beet salad: Beet; Vinaigrette (oil, salt and vinegar).

C. Green salad: Green salad;Tomato; Vinaigrette (oil, salt and vinegar).

D. Chicken: Chicken; Oil; Spices: salt, cumin, black pepper and saffron.

E. Kosher: packaged marketed product.

F. Dessert Juice

Methods. We carried out two methods to carry out our study in order to verify the rules of hygiene:

 Methods of microbiological analysis;

 Methods of application of HACCP.

Microbiological analysis method. We carried out the microbiological analyzes at the level of the hygiene laboratory in Saida, the CACQE laboratory in Saida and the microbiology laboratory at the level of our Biology department.

Microbiological analysis of food (the selected cooked dish). Microbiological analysis of the preparation water. We carried out microbiological analysis of the preparation water at the Saida hygiene laboratory.

 Sample collection

For the microbiological analyses, the sample was taken according to the following steps:

 Clean the tap and disinfect it by flame;

 Prepare clean glass bottles for sampling;

 Open the sampling valve and allow water to flow for at least 2 minutes before collection;

 Uncork the bottle, flambé its neck;

 Fill the bottle slowly at a low rate to avoid turbulence;

Release the cap from its casing;

 Close and label the sample bottles determining the sampling point, date and time.

Microbiological analysis of the surface and material. We took the sample using the wet swab method:

 Moisten the swabs with sterile distilled water;

 Rub the swab on the surface and the material;

 Inoculate on the culture media already poured into the petrie dishes.

We carried out the control of the surfaces "after cleaning" on:

 Kitchen wall;

 Kitchen floor;

 The bench of the distribution chain;

 Wall of the butchery;

 Floor of the butchery;

 The butcher's table.

Concerning the control of the material we carried out the sampling on:

 A cooked dish and the knife "after cleaning"

Microbiological analysis of the air. We carried out the air control on the following media:

 "Positive" storage chamber; Food storage room; The kitchen and the butcher's room.

Method of application of HACCP. Our experiment involves the application of the HACCP

system at the level of the central university catering in Saïda. Our study took place during the period of January - March 2022.

HACCP steps. -Constitution of the HACCP team

The HACCP team was structured as follows: The project supervisor; Trainees: two students enrolled in the “master 2” applied microbiology program; The head of the catering service (Biologist); The chef.

-Field of study

 Product of the "cooked meal" study;

 The nature of the microbiological hazards;

 Level of application: from the receipt of raw materials to the release for consumption of ready meals.

-Description of the product (cooked meal)

We chose the first dish of the week consisting of:

 Lentil soup; Beet salad; Green salad; Chicken; Kosher; Juice.

Expected use. Consumption by students as a hot meal (on site and at mealtimes);

The consumption of cooked dishes must allow an energy balance, the dishes will be consumed preferably at a temperature of 35°C-50°C;

On-site verification of manufacturing diagram. This step consists of on-site verification of the preparation diagram for the lentil, chicken, beet salad, green salad, kosher and juice soup.

We compared the flow of activities to the diagram of operations during working hours.

Washing; cutting and draining of the material are carried out in poor hygienic conditions.

TABLE 2
CCPs for the preparation of lentil soup

Step 1						
Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Receiving the ingredient ″onion ″	Yes	Yes			CCP	Contamination step
Storage	Yes	Yes			CCP	Contamination step
Onion washing and cutting	Yes	Yes			CCP	Contamination step
Add oil + spices + concentrated tomato + salt	Yes	No	No		No CCP	No contamination step
Cooking	Yes	No	Yes	Yes	No CCP	No contamination step
Add water	Yes	No	No		No CCP	No contamination step
Cooking	Yes	No	Yes	Oui	No CCP	No contamination step
Transfer	No	No			No CCP	No contamination step
Step 2						
Decision tree Critical point	**Q1**	**Q2**	**Q3**	**Q4**	**CCP**	**Observation**
Reception of potato and carrot vegetables	Yes	Yes			CCP	contamination step
Storage	Yes	Yes			CCP	contamination step
Potato and carrot washing and cutting	Yes	Yes			CCP	contamination step
Transfer	No	No			No CCP	No contamination step
Step 3						
Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Reception of the 1st “lentils” material	No	No			No CCP	No contamination step
Storage	No	No			No CCP	No contamination step
Lentils sorting	Oui	Oui			CCP	contamination step
Lentils washing	Oui	Oui			CCP	contamination step
Transferring lentils to the pot	Oui	No	No		No CCP	No contamination step
Adding water	Oui	No	No		No CCP	No contamination step
Cooking	Oui	No	Oui	Oui	No CCP	No contamination step
Distribution	Oui	Oui			CCP	contamination step

TABLE 3
The CCPs of the preparation of beetroot salad

Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Reception of the raw material "beet-root"	yes	Yes			CCP	contamination step
Storage	yes	Yes			CCP	contamination step
Washing	yes	Yes			CCP	contamination step
Cooking	yes	No	Yes	yes	no CCP	No contamination step
Cutting into small squares	yes	Yes			CCP	contamination step
Transferring to a dish	No	No			no CCP	No contamination step
Addition of dressing	yes	No	No		No CCP	No contamination step
Distribution	yes	Yes			CCP	contamination step

TABLE 4
The CCPs of the preparation of the green salad

Step 1

Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Receipt of the 1st ingredient ″to-mato″	yes	Yes			CCP	contamination step
Storage	yes	Yes			CCP	contamination step
Washing	yes	Yes			CCP	contamination step
Cutting	yes	Yes			CCP	contamination step
Transfer	No	No			no CCP	No contamination step

Step 2

Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Receipt of the raw material ″green salad″	yes	yes			CCP	contamination step
Storage	yes	yes			CCP	contamination step
Cutting	yes	yes			CCP	contamination step
Transfer to a large pool	No	No			no CCP	No contamination step
Wash 2 times with water and bleach	yes	yes			CCP	contamination step
Drain in a large colander	yes	No	No		no CCP	No contamination step
Transfer to a large container	Non	No			no CCP	No contamination step
Addition of dressing	yes	No	No		no CCP	No contamination step
Distribution	yes	yes			CCP	contamination step

TABLE 5
Chicken preparation CCPs.

Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Reception of the raw material ″chicken″	yes	yes			CCP	contamination step
Washing	yes	yes			CCP	contamination step
Cut into pieces	yes	yes			CCP	contamination step
Transfer to a dish	yes	yes			CCP	contamination step
Addition of oil, salt, cumin, black pepper and saffron	yes	Non	Non		No CCP	No contamination step
Cooking 300°C /45min	yes	Non	yes	yes	No CCP	No contamination step
Distribution	yes	yes			CCP	contamination step

Hazard analysis. The hazards may constitute CCPs, are determined in several ways:

 The dangers related to the lack of hygiene (equipment and food items);

 The dangers related to the storage conditions (time and Temperature);

Microbiological hazards. To identify the hazards, we used the "5M" method. The material: represented the initial source of food contamination;

 Equipment: represents the different equipment used in catering: knives, cooked dishes, pots, robots, ladles, strainers and containers;

 The environment: represented the premises and the surfaces for preparing and cutting the distribution;

 The method: consists of analyzing the preparation, the handling and the distribution operations;

The workforce: represented by the people who are in the preparation site (waiters and cooks).

Among the sources of microbial contamination are: hands, clothes and hair.

Determine the critical points for the control of CCP hazards

CCPs (Critical Control Points) are essential to prevent, eliminate or reduce a food safety risk to an acceptable level.

We used the decision tree to check the possible CCPs of the different stages of the ready meal preparation diagram (see Tables 2, 3, 4, 5,6 and 7).

Establish critical limits for each CCP. Critical limits are criteria that help distinguish safe products from unsafe products. In our study we determined critical limits for each of the CCPs, and are represented in Tables 2,3, 4,5,6,7.

Establish a monitoring system for each CCP. Consists of performing a series of observations or measurements to determine if a CCP has been controlled.

The existence of a representative dish representative of each dish consumed at the necks of the week, the latter is kept in a special refrigerator at a temperature of 4°C for 3 days.

Establish corrective actions. Specific pre-established activities must be planned for each CCP to ensure risk control. We have summarized the corrective actions in Tables 8 and 9.

Establish a recording and documentation system. Like any quality approach, HACCP uses the records that are necessary. Two types of documents can be distinguished:

Documentation on the implementation: procedures, operating modes, work instructions, these documents constitute the "HACCP plan"

Records, results, observation, reports, records of decisions.

Establish verification procedures. Verification confirms that the HACCP plan is working effectively.

We performed microbiological analyzes on the selected cooked dish, surface, air, materials and preparation water for verification.

RESULTS AND DISCUSSION

Results and interpretations of microbiological analyzes. Results and interpretations of the microbiological analyzes of the selected cooked dish. The results obtained (see tables 10, 11, 12, 13, 14 and 15) are compared with the national standards of the Official Journal of the Algerian Republic N°39 Interministerial decree of 2 Muharram 1438 corresponding to October 4, 2016 setting the microbiology criteria of food items [12].

The number of colonies obtained on each specific medium is expressed in CFU/g for the five samples analyzed.

TABLE 6
CCPs of kosher preparation

Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Reception of the 1st ″kosher″ material	yes	No	No		No CCP	No contamination step
Storage	yes	yes			CCP	contamination step
Cutting	yes	yes			CCP	contamination step
Distribution	yes	yes			CCP	contamination step

TABLE 7
Juice preparation CCPs

Decision tree Critical point	Q1	Q2	Q3	Q4	CCP	Observation
Reception of the raw material "juice"	Yes	No	No		No CCP	No contamination step
Storage	Yes	Yes			CCP	contamination step
Distribution	No	No			No CCP	No contamination step

TABLE 8
Results of microbiological analysis of lettuce

Germs wanted	Results	Microbiological limits (ufc/g)	
Yeast and mold	3.10^3	10	10^2
Aerobic germs	Uncountable colony	3.10^5	3.10^6
Staphylococci aureus	2.10^3	10^2	10^3
Clostridium sulphite-reducer	Uncountable colony	50	5.10^2
Enterobacteriaceae	C Yellow C Purple C Red	10	10^2

TABLE 9
Results of microbiological analysis of the preparation water

Germs wanted	Results	Microbiological limits (ufc/g)
Total coliforms	Negative results	
Faecal coliform	Negative results	
Faecal streptococcus	Negative results	

TABLE 10
The results of the microbiological analyzes of the surfaces analyzed

Surfaces analyzed	***Culture medium used***	**Number of colonies**	**Appearance & color of colonies**
	GN	79	
The butcher's wall	SAB	4	Small size in White and yellow color
	Hectoen	No colonies	Large size in White color
	Chapman	26	/
	VF	Uncountable	Big and small size in White and yellow color
The floor of the butcher's shop	GN	Uncountable	Large size in White and yellow color
	SAB	Uncountable	Large size in White color
	Hectoen	12	Bench and yellow color large size
	Chapman	Uncountable	Small size in White and red color
	VF	Uncountable	Large size in Military Green color
The butcher's table	GN	/	Big and small size in White and yellow color
	SAB	Uncountable	Small size in White color
	Hectoen	/	/
	Chapman	Uncountable	Small size in White and yellow color
	VF	/	Small size in White color
The kitchen wall	GN	10	Big and small size in White color
	SAB	4	Large size in White color
	Hectoen	No colonies	/
	Chapman	4	Large size in White color
	VF	Uncountable	Large size in White color
The kitchen floor	GN	Uncountable	small size in White color
	SAB	Uncountable	Big and small size in White and yellow color
	Hectoen	No colonies	/
	Chapman	16	Big and small size in White color
	VF	Uncountable	Large size in White color
	GN	Uncountable	
	SAB	Uncountable	Big and small size in White and yellow color
	Hectoen	No colonies	Big and small size in White color
	Chapman	50	/
	VF	Uncountable	Big and small size in White and yellow color

TABLE 11
Results of microbiological analyzes of materials

Materials analyzed	*Culture medium used*	Number of colonies	Appearance and color of colonies
The dish	GN	Uncountable	Small size in White and yellow color
	SAB	Uncountable	Small size in White color
	Hectoen	No colonies	/
	Chapman	45	Small size in White color
	VF	Uncountable	Big and small size in White color
Knives	GN	Uncountable	Big and small size in White and yellow color
	SAB	Uncountable	large size in white and yellow color
	Hectoen	9	large size in Black color
		Uncountable	large size in yellow color
		Uncountable	large size in Red color
	Chapman	26	Big and small size in White color
	VF	Uncountable	Small size in White color

No observed value exceeds the "M" limit. So the results of microbiological analysis of lentil soup, beetroot salad, chicken, kosher and juice are satisfactory and of accepted quality for the health of students

According to the analysis results mentioned in table 8, we noted the presence of yeasts, molds, aerobic germs, Staphylococci aureus and Clostridium Sulfito-reducer, so this non-compliance with standards is mainly due to the lack of equipment, personnel and air hygiene. According to Belomaria *et al*, 2007; Durieux; 1978)_[13], [14] a poor hygienic quality of lettuce results mainly from a lack of hygiene and non-compliance with good practices, is the cause of a large number of food poisoning cases.

Results and interpretations of microbiological analyzes of the preparation water. We compared our results obtained with the ISO standard, according to Table 9, the results of the preparation water analysis comply with the standards

Results and interpretations of microbiological analyzes of surfaces.

TABLE 12
Results of microbiological air analyzes

Media analyzed	*Culture medium used*	Number of colonies	Appearance and color of colonies
The cold room	GN	20	Small size in White color
	SAB	No colonies	/
	Hectoen	No colonies	/
	Chapman	3	small size in White color
	VF	No colonies	/
The feeding chamber	GN	10	small size in yellow color
	SAB	No colonies	/
	Hectoen	No colonies	/
	Chapman	1	small size in yellow color
	VF	3	Large size in color White surrounded by black
The butcher's shop	GN	Uncountable	Small size in White color
	SAB	2	Large size in White color
	Hectoen	1	Small size in Military Green color
	Chapman	5	Small size in Yellow color
	VF	No colonies	/
The kitchen	GN	40	Small size in White and yellow color
	SAB	1	Large size in White color
	Hectoen	8	size small in Military Green color
	Chapman	11	small size in white and yellow color
	VF	No colonies	/

TABLE 13
HACCP control "lentil soup" step 1

Step 1	Hazards	Preventive measures	Critical Limits	Surveillance	Corrective actions
Receipt of the first ingredient ″onion ″	Microbial contamination	Control of the separation between food products	Correct conditions From reception	Visual control	Control before receipt from the supplier
Storage	Microbial contamination	Temperature and humidity control	Correct conditions From reception Temperature, wet weather	Visual control	Repair of the vegetable room (ventilation system)
Washing and cutting	Microbial contamination (surface, equipment and personnel)	Cleaning procedure		Visual control	Cutting room addition
Addition of oil+spices=tomato paste+salt	No dangers			Visual control	
Cooking	No hazards				
Add water	No hazards				
Cooking	No hazards				
Transfer	No hazards				

TABLE 14
HACCP control "lentil soup" step 2

Step 2	Hazards	Preventive measures	Critical Limits	surveillance	Corrective actions
Preparing the vegetables	Microbial contamination	Control of the separation between food products	Correct conditions From reception	Visual control	Control before receipt from the supplier
Storage	Microbial contamination	Temperature and humidity control	Correct conditions From reception Temperature, wet weather	Visual control	Repair of the vegetable room (ventilation system)
Washing and cutting	Microbial contamination (surface, equipment and personnel)	Cleaning procedure		Visual control	Cutting room addition
Transfer	No hazards				

Results and interpretation of microbiological analyzes of materials. Microbiological analyzes of the work surface and equipment (see Tables 10 and 11) reveal an increase in fungal flora and other pathogenic germs which indicates that:

 The washing of the dish and the knife is done in poor hygienic conditions ;

The cleaning of the bench, floor, wall and butcher's table does not comply with hygiene rules.

The presence of yeast on the surface of foods can, in some cases, lead to spoilage of these foods. When they grow on food in too high a concentration, taste, texture and appearance can be impaired [15], [16].

Results and interpretations of microbiological air analyzes. From Table 12, we have seen an increase:

Total mesophilic aerobic flora, this is favored by several factors such as: Temperature, and humidity, which causes organoleptic changes in foods;

Some pathogenic germs. The prevention of contamination requires good organization of work, in order to limit and manage the trips of staff to the waste room. The evacuation of the waste room must be done outside the period of preparation of the dishes in the kitchen and before the disinfection of the premises [17], [18]. The air can be loaded with microorganisms responsible for deterioration or even disease. Indeed, airborne dust and particles are likely to contaminate work surfaces as well as food [19]

Results and interpretations of the HACCP system. The results obtained are mentioned in tables 13,14 and 15:

From tables 13,14 and 15, we noticed that:

The steps in the preparation of the selected cooked dish represent microbiological hazards; these dangers are mainly due to:

 Lack of application of good hygiene practices; Storage of non-compliant product; Contamination by personnel, surfaces and materials.

We have proposed a few "corrective actions" for each stage of preparation and distribution of the selected cooked meal.

Our study aimed to apply the HACCP system and to estimate the good hygiene practice at the level of the central university catering in Saida.

This study showed that it had a set of failures:

 Non-compliance with hygiene rules, in particular: - During the preparation of cooked meals; In premises, surfaces, equipment and the air, Personal; no ventilation system in storage rooms; Un qualified personnel. The HACCP system takes into account all the potential risks and factors that can harm the health of the consumer and is also applied to the determination of the critical control points necessary to control the dangers [20], [21], [22].

CONCLUSION

This study exposed a set of failures:

 Non-compliance with hygiene rules, in particular :

 During the preparation of cooked meals ;

 In premises, surfaces, equipment and the air

TABLE 15
HACCP control "lentil soup" step 3

Step 3	Hazards	Preventive measures	Critical Limits	surveillance	Corrective actions
Receipt of "lens"	No hazards	Control of the separation between food products	Correct conditions From reception	Visual control	
Storage	No hazards	Temperature and humidity control	Correct conditions From reception Temperature, wet weather	Visual control	
Lens sorting	Physical danger presence of foreign bodies (glass; stones) Contamination of personnel and equipment	Sanitary condition cleaning of equipment	Cleaning procedure	Visual control	Staff training
Lens washing	Microbial contamination (surface, equipment and personnel)		Correct hygienic conditions		Respect hygiene rules
Transfert Addition of water	No hazards				
Cooking	No hazards				
Distribution	Contamination microbial (surface; servers)	Staff hygiene and disinfection of equipment	Medical examination Perfect cleanliness	Visual and microbiological control	Staff training and application of hygiene rules Carriages

- Personal;
- No ventilation system in the storage rooms;
- Unqualified personnel.

These bad practices are characterized by the presence of spoilage germs, faecal contamination germs and mainly Escherichia coli and fungal flora in food.

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DEGRADATION OF VOLATILE ORGANIC COMPOUNDS USING MICRO / NANOROD MOLECULAR SIEVE IN A COMBINED REACTOR

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ABSTRACT

The purpose of this study is to prepare the micro/nanorod materials in low middle temperature to remove the volatile organic compound (taking toluene as target) in the waste air pollution stream using a combined reactor with photo- and thermo-reaction at the same time. The micro/nanorod molecular sieve (Octahedral manganese cryptomelanes, K-OMS-2) are fabricated and coated on support of Al_2O_3, and the catalyst is used in the combined reactor with light and heat sources at feasible temperature to convert the target. The decomposition/removal efficiency of toluene in photo- and thermos-reactor as well as the photoelectric characteristic analyses are evaluated. The operation parameters of temperature of reactor and initial concentrations etc. are carried out, meanwhile, the characteristic analyses (EA, TEM-EDS, XRD, LaB6-TEM, XPS, FTIR etc.) are also examined in order to find the optimum conditions. In the performance of the photodegradation reaction, the optimum η is the catalyst of K-OMS-2/Al_2O_3 calcination temperature of 523 K with UVLED of 633 hr^{-1} space velocity (SV), and the maximum value is 95.56%. However, the CO_2 conversion is not obvious in the photodegradation reaction due to the not complete degradation. In the photo- and thermos-degradation reactor, the situation can be improved and the maximum η of 95.25% and CO_2 conversion of 95% can reach at 873K with UVLED. The result proves that the combined reactor for photo- and thermos-degradation can work in both industrial or domestic application in air pollution control.

KEYWORDS:
Nanorod molecular sieve, thermoelectric material, photo-thermo-catalyst, Octahedral manganese cryptomelanes, air pollutant, photo-thermal-catalytic reaction.

INTRODUCTION

The report of the World Health Organization in 2014 showed that 7 million deaths worldwide were related to air pollution in 2012. With the rapid development of industry and commerce, a large number of volatile organic gases (VOCs) are emitted into the atmosphere during the operation of factories, such as benzene, toluene, ethylbenzene, xylene, and other BETXs, which are mainly used as solvents and adhesives in the plastics and tire industries, printing houses, and various chemical plants; however, these gases will cause the deterioration of the working environment and the surrounding air quality, further affecting the health of factory employees and nearby residents.

Photodegradation is a common degradation method to remove air pollutants. Catalysts that use light energy to catalyze the reaction are called photocatalysts, which will not be consumed by participating in the reaction and can continuously catalyze the degradation reaction. Under light irradiation, it can be excited to generate electron–hole pairs, which can react with water and oxygen to generate hydroxyl radicals, so as to decompose various volatile organic compounds (VOCs). A molecular sieve is a material with numerous small pores of precise and uniform size. These pores are large enough to stop macromolecules but allow small molecules with pore sizes falling within the specified range to pass through. Common molecular sieves include desiccant, activated carbon, and silica gel. According to the International Union of Pure and Applied Chemistry (IUPAC), porous materials are classified into three categories by the pore diameter, namely micropores, mesopores, and macropores. Mesoporous molecular sieves have pore sizes ranging from 2 to 50 nm and have the advantages of high specific surface area, uniform pore distribution, and application in the technical fields of catalysis and separation, such as MCM-41[1], HMS [2], MnO_2-based catalysts [3, 4] and SBA-15 [5].

Manganese is a transition metal that occurs widely in nature (the tenth most abundant element in the earth's crust) [6]. The nano-sized manganese metal oxides have many advantages, such as large specific surface area, porous structure, many active sites, good thermal stability, easy recovery, high environmental compatibility, and low toxicity [7, 8]. It is pointed out in the literature that the cryptomelane-type manganese oxide-based octahedral molecular sieve (K-OMS-2) made of micro/nanorod material has great benefits for catalytic activity, so the application of K-OMS-2 in catalytic oxidation technology is widely studied [9]. Some cations, such as Li^+, K^+, and Na^+, can enter the channel of K-OMS-2 to adjust their chemical and physical properties [10]. It substantially influences the catalytic activity by adding different concentrations of K^+ into OMS-2 to determine the most appropriate dosage [11, 12]. Studies have proposed that K-OMS-2 is implanted onto TiO_2 to form K-OMS-2/TiO_2 [13] and toluene is degraded under the photodegradation by using a ultraviolet (UV) lamp, which has a better result than that of commercial TiO_2. The hierarchically magnetic K-OMS-2/TiO_2/Fe_3O_4 heterojunction exhibits good photocatalytic activity for the degradation of humic acid under solar light [14]. The reasons are that it can form OH functional groups on the catalyst surface to assist the adsorption and decomposition of toluene, so K-OMS-2 can also be regarded as a molecular sieve photocatalyst [15].

MATERIALS AND METHODS

Material preparation. Preparation process of OMS-2. OMS-2 was dissolved in D.I. water by combining $MnSO_4$, H_2O, $(NH_4)_2S_2O_8$, and $(NH_4)_2SO_4$. After being fully dissolved, the mixture was calcined at 393 K in a high-temperature and high-pressure reaction reactor for 20 hours. The high-pressure reaction reactor was used for solid-liquid separation after being cooled to room temperature, and the solid is dried through 4-hour illumination by the infrared lamp and then ground for 40 minutes to complete the preparation of OMS-2.

Preparation process of K-OMS-2. $MnSO_4 \cdot H_2O$ 2.3521 g, $(NH_4)_2S_2O_8$ 1.8256 g, and $(NH_4)_2SO_4$ 1.9821 g were added into D.I. water for mixing for 10 minutes, and then KNO_3 was added for dissolution to form the mixture. Finally, the mixture was calcined at 393 K in a high-temperature and high-pressure reaction kettle for 20 hours. The high-pressure reaction kettle was cooled to room temperature for solid-liquid separation, dried by 4-hour illumination of the infrared lamp, and then ground for 40 minutes to complete the preparation of K-OMS-2.

Preparation catalyst process of K-OMS-2/Al2O3. Take a proper amount of K-OMS-2 and Al2O3 in alcohol, heat, stir, and soak them with a magnetic stirrer, and then put them in a dryer for drying to complete the preparation of K-OMS-2/Al_2O_3 catalyst.

Characteristic analyses of photocatalysts. The sample mass used was 0.5 g and dried for 24 h before characteristic analyses. The analysis instruments used were thermal field emission scanning electron microscope (FESEM) (JSM-7000F), transmission electron microscope (LaB6-TEM /FEI Tecnai G2 T20 200kV), energy dispersive spectrometer (EDS) (LEO 1530, Germany), element analyzer (EA) (Elementar VarioEL-III, Germany), Brunauer Emmett Teller (BET) surface measurement device (Micromeritics ASAP2020, Micromeritics, USA), X-ray single-crystal diffractometer (XRD) (Rigaku TTRAX Ⅲ, Japan), X-ray photoelectron spectroscopy analyzer (XPS) (VG MICROTECH, MT-500, UK), ultraviolet-visible (UV/Vis) spectroscopy (UV/Vis spectrophotometer, EVOLUTION-220, Thermo Scientific, USA) and Fourier transform infrared spectrometry (FTIR) (Thermo Fisher IS10), photoluminescence spectrum (PL) (JOBIN-YVON T64000 Micro-PL/Raman Spectroscopy), Thermal Image Camera (Thermo Gear G100EX / G120EInfrared Camera).

Thermo-Photo-Degeneration Characteristics Experimental preparation of thermo- photodegradation. Target pollutants. Toluene was target air pollutant in this study. It was prepared by transporting saturated toluene vapor through a stable airflow into a mixing tank for reaction and then into a continuous first-stage reactor for photodegradation reaction.

Characteristics of light intensity for Thermo-photo-degradation. The light sources used in this experiment were T8UVALED, T8WLED (color temperature 5,600–6,300 K), and T8BLED sold by vita LED Technologies Inc. Their light source characteristics are as follows Table 1. Measure the light source intensity of the first-stage photodegradation reaction lighting box at different positions, and the average illuminance could be obtained after the light source characteristics were corrected, as shown in Table S1 and Table S2.

Thermo-photo-degradation apparatus. In this study, a two-stage degradation reaction system was established, which was configured with a programmable tubular high-temperature furnace and a lighting box, the former of which exhibited the programmable temperature control function. A 4-inch stainless steel tube (inner diameter: 95 mm, outer diameter: 100 mm, length: 1,000 mm) in the programmable tubular high-temperature furnace body was connected to a 2-inch quartz tube (inner diameter: 46

mm, outer diameter: 50 mm, length: 1,000 mm) in the lighting box body, as shown in Figure 1. The incoming gas could flow into the lighting box for photodegradation after being heated by the programmable tubular high-temperature furnace.

In the first-stage photodegradation, the mass flowmeter of the photodegradation reaction system was set to be 1 L/min for incoming air, and the float flowmeter was set to be 0.06 L/min for incoming toluene. Turn on the lamp source when the concentration was stable after 35 minutes for sampling, and collect samples at 10–19, 20–29, 30–39, 40–49, and 50–59 minutes after the light was turned on, representing the average concentration at 15, 25, 35, 45, and 55 minutes, respectively. Use a 1 L gas sampling bag to collect the gas after the reaction. With an inflation needle, 150 μL gas was extracted from the sampling bag and injected into a gas chromatography mass spectrometer (GC-MS) for conversion of toluene concentration. The models of GC-MS included Thermo Scientific Focus GC and DSQ 12550080.

In the second-stage thermo-photo-degradation, the mass flowmeter of the photodegradation reaction system was set to be 1 L/min for incoming air. The float flowmeter was set to be 0.06 L/min for incoming toluene, and the heating box was turned on. The temperature in the box shall reach 423, 473, 573, 673, 773, 873, 973, and 1,073 K, respectively, within 35 minutes. After the gas concentration was stable, the lamp source was turned on for sampling. Samples were collected at 56–65 minutes after the light was on, representing samples of 1 hour after the thermo-photo-degradation. A 1 L gas sampling bag was used to collect the gas after the reaction. 150 μL gas was extracted from the sampling bag using an inflation needle and then injected into a gas chromatography mass spectrometer (GC-MS) for the conversion of toluene concentration or into a gas chromatograph-thermal conductivity detector (GC-TCD) for the analysis of the catalyst CO_2 conversion rate, with GC-TCD models including the Thermo Scientific Focus GC and DSQ 12550090.

TABLE 1
Characteristics of light sources used in this study.

	Viewing angle	Power (W)	WL[d] range (nm)	llumination (Lux)
WLED[a]	120°	17	300~700	13,520
UVLED[b]	90°	17	380-390	129
BLED[c]	120°	17	465-475	1,399

[a].White visible light emitting diode. [b.] Ultraviolet light emitting diode; [c]. Blue light emitting diode [d]. Wavelength.

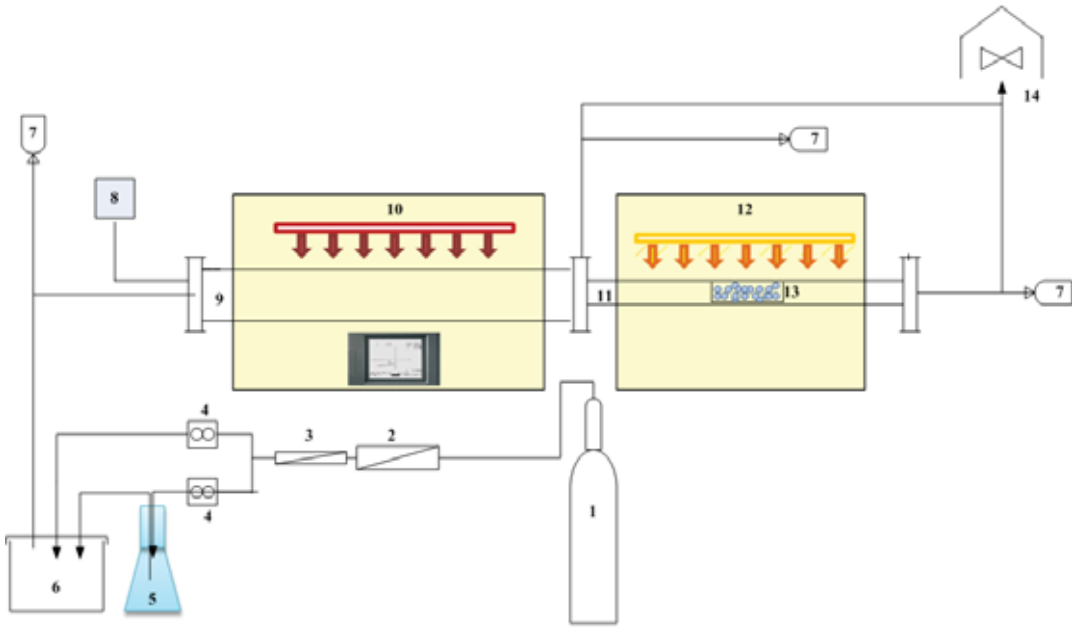

FIGURE 1
Schematic of the hybrid system of the thermodegradation and photodegradation reactors.
1. Air cylinder, 2. Moisture removing trap, 3. HC removing trap, 4. Mass flow controller, 5. Toluene liquid Erlenmeyer flask, 6. Mixing box, 7. Sample output apparatus, 8. Thermometer, 9. Stainless steel vacuum sleeve for diameter of 4″, 10. Muffle furnace, 11. Stainless steel vacuum sleeve for diameter of 2”, 12. Light source, 13. Catalyst bed, and 14. Fume hood.

TABLE 2
EA[a] and EDS analysis of copper modified photocatalysts[b].

Sample	EA					EDS		
	N	C	H	S	O	K	Mn	Si
SBA-12	0.37	0.46	1.13	-	65.88	-	-	32.15
OMS-2	9.48	0.11	2.8	7.76	61.16	-	20.20	-
K-OMS-2(393K)	4.79	0.08	1.3	3.30	62.24	2.55	27.70	-
K-OMS-2(423K)	7.61	0.09	2.18	9.81	36.50	6.69	37.12	
K-OMS-2(523K)	2.61	0.09	0.86	13.71	40.37	9.18	33.17	
K-OMS-2(623K)	1.76	0.09	0.53	18.44	41.34	11.07	26.78	-

[a]. Accuracy: ±0.1%. Precision: ±0.2%. [b]. unit: wt.%

RESULTS AND DISCUSSION

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Characteristic analyses of photocatalyst materials. Energy dispersive spectrometer (EDS) and element analyzer (EA). TEM-EDS can qualitatively and semi-quantitatively analyze the elements on the catalyst surface, so that the content of the elements can be known by measuring the intensity of spectral lines. In this study, TEM-EDS was used to analyze the surface elements, from which it could be known that K-OMS-2 was indeed covered with K. The analysis result is shown in Table 2. The weight percentages of N, C, H, O, S, K, and Mn in OMS-2 and K-OMS-2 were detected by EA and EDS. Because EDS could not analyze C and H, EA was obtained first to analyze the sum ratio of N, C, and H, and then the individual percentage measured by EDS was divided by the remaining percentage of EA to obtain the weight percentage of O, S, K, and Mn. The results showed that K, O, and Mn increased in K-OMS-2 compared with those in OMS-2, and the decrease of N% content may be due to the substitution of N by other ions. The bond of N could be determined by other instruments later.

It can be seen from the data that the increase in K, O, and Mn would lead to a decrease of C, H, and S contents. After K was covered, O content increased from 61.16% to 62.24%, S content decreased from 7.76% to 3.3%, and Mn content increased from 20.2% to 27.7%, showing that the changes of these elements were relatively greater than those of other elements. After K-OMS-2 was calcined at 423, 523, and 623 K, the results indicated that with the increase in calcination temperature, O content would first decrease and then increase, with the content decreasing by approximately 21%–26%; S and K contents would increase by 6.48%–15.11% and 4.14%–8.52%, respectively, with the increase in temperature. Mn content would first increase and then decrease. After K-OMS-2 was calcined at 423 K and 523 K, Mn content would decrease by 9.42% and 5.47%. After K-OMS-2 was calcined at 623 K, the Mn content would decrease by 0.92%. The changes in K and Mn content indicated that when the calcination temperatures were 423 K and 523 K, both K and Mn contents increased, while K content at 523 K was higher than that at 423 K.

X-ray diffractometer (XRD). The catalyst was analyzed using X-ray diffraction (XRD). Compared with the database of the Joint Committee on Powder Diffraction Standard and the International Centre for Diffraction Data, Figure S1(a) is the diffraction pattern of SBA-15. A broad wave peak was identified at $2\theta = 15°–30°$ due to its mesoporous structure. OMS-2 (Figure S1 b)) and K-OMS-2 (Figure S1 (c)) exhibited the same pattern of the synthetic material $K1.49(NH_4)0.51Mn_2(SO_4)_3$ of cryptomelane. OMS-2 and K-OMS-2 showed that there was an obvious wave peak at 25°–30°, which was consistent with the crystalline phase of Pyrolusite MnO_2 [3, 16].

The ultraviolet–visible spectroscopy (UV/Vis). Liua et al. (2011) proposed that K-OMS-2 exhibited a significant absorption value in the zone of about 400 nm [17]. Compared with the results of UV/Vis analysis in this study, as shown in Figure S2, K-OMS-2 and OMS-2 had favorable absorption values than that of SBA-15 in the excellent absorption zones for ultraviolet light and visible light, while K-OMS-2 exhibited a lower absorption value than that of SBA-15 in the zone of above 500 nm. The optimal absorption value was approximately 350–500 nm, falling in the zone between ultraviolet to visible light. The absorption value of K-OMS-2 was slightly improved after being calcined. The absorption zone of cryptomelane was the overlap zone of different valence states of manganese, namely, Mn^{2+}, Mn^{3+}, and Mn^{4+} in manganese crystal. The zone at about 250 nm was formed by charge transfer between O_2 and Mn^{2+}, and the zone at about 300 nm was connected with Mn^{4+}. The continuous absorption of the zone depended on $O_2 \rightarrow Mn^{3+}$ charge transfer and d-d conversion into the d4 electron configuration [18, 19].

Photoluminescence spectrum analyzer (Micro-PL). Micro-PL was used to analyze the influence of catalyst structure. Because K-OMS-2/Al_2O_3 was solid, the powder must be used in Micro-PL

measurements; therefore, K-OMS-2 powder was calcined at various temperatures in this study to measure its Micro-PL. From Figure 2(a), the peak value at 495 nm^{-1} was the highest in the pattern, and the luminous intensity order was K-OMS-2 > SBA-15 > OMS-2. The FWHM of the peak showed that the smaller the FWHM, the less impact on the sample from impurities and defects, and the crystallization was relatively ideal, so the luminous intensity of OMS-2 was relatively weak but the crystallization was optimal. As for K-OMS-2 calcined at different calcination temperatures in Figure 2(b), K-OMS-2(523K) exhibited the best crystallization, while the luminous intensity was the opposite. However, the luminous intensity of K-OMS-2 at different calcination temperatures was above a certain level. The pattern showed that the peak value of the catalyst at 415 and 526 nm^{-1} was contributed by the stretching vibration of the Mn-O lattice, representing the 2×2 pore tunnel structure, and the external vibration caused by the parallel movement of the octahedron [20-22], in which when the central luminescent ion is Mn^{2+} or Mn^{4+}, the corresponding transition is 3p→3d [21].

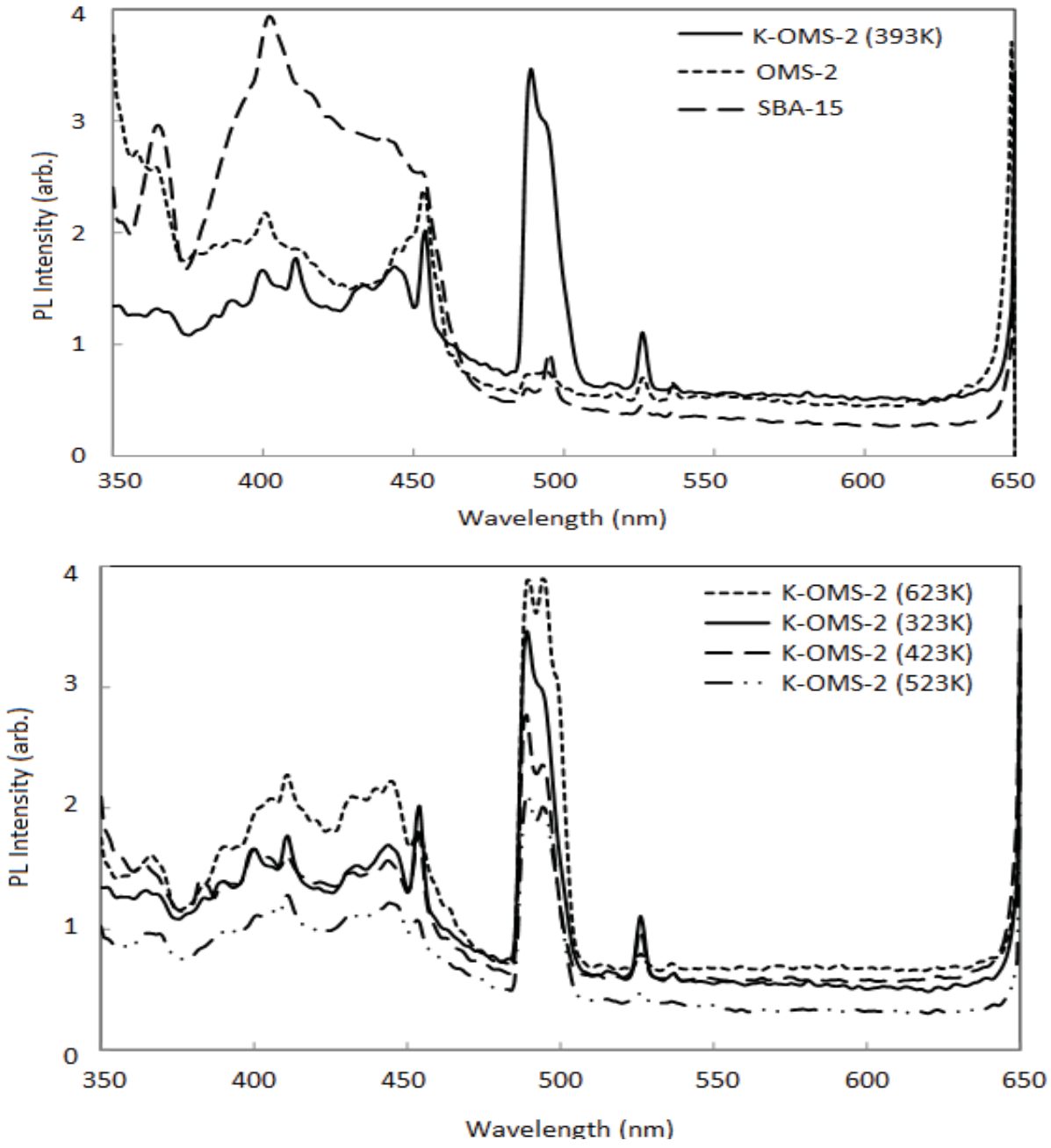

FIGURE 2
Micro-PL spectra of (a)K-OMS-2 (393K), OMS-2 and SBA-15 (b) K-OMS-2 in different calculation Temps.

The Brunauer, Emmett, and Teller (BET) analysis. Table 3 indicated the influence of different calcination temperatures on the pore structure of the catalyst based on the analysis and judgment of BET. The specific surface area of K-OMS-2/Al_2O_3 is 523 K>423 K>623 K, representing 279.78, 270.78, and 265.43 m^2/g, respectively. Therefore, the BET of K-OMS-2/Al_2O_3 catalyst calcined at 523 K was the largest, and the calcination temperature was speculated to cause the change of the pores of K-OMS-2/Al_2O_3, affecting the surface area. The low calcination temperature was insufficient to increase the surface area of catalysts, while the relatively high calcination temperature may lower the surface area. The average adsorption pore diameter of K-OMS-2/Al_2O_3 calcined at 423, 523, and 623 K is 51.82, 51.72, and 52.22 Å, and the average desorption pore diameter was 42.97, 43.38, and 43.67 Å, respectively. This indicated that no matter the average pore diameter for adsorption or desorption, the influence of the pore diameter difference at different calcination temperature was not obvious, and their standard deviations were lower than 1.

Fourier Transform Infrared Spectrometry (FTIR). FTIR pattern (Figure 3) showed that SBA-15 exhibited functional group primary aliphatic alcohols. Compounds with (R–CH_2–OH) double bonds and hydroxyl (–OH) groups were classified as primary, secondary, or tertiary according to the number of carbon atoms attached to the surface of the oxygen-bonded carbon. Primary aliphatic alcohols contained polar –OH groups. Because the boiling point of this hydrogen-bonded alcohol was much higher than that of corresponding alkanes with the same number of carbon atoms, hydrogen was bonded between molecules in the condensation phase. OMS-2 showed the Kaolin clays/alumino silicate bond at 1,021, 1,103, and 1,413 cm^{-1} peaks, while –OH bond at 3,211 cm^{-1}. In addition to the Kaolin clays/alumino silicates at the K-OMS-2 peaks of 931, 1,092, and 1,419 cm^{-1}, a para substituted aromatic hydrocarbon (para substituted aromatic hydrocarbon) functional group was added, and –OH bond was formed at 3,180 cm^{-1} (Figure 8 (c)). The characteristic analysis of EA and EDS indicated that C content and H content would increase after K-OMS-2 was calcined at 523 K, which may be the reason for the formation of aliphatic groups. The most essential vibration mode of this group was C–H of approximately 3,000 cm^{-1} and –CH extending to about 1,460 cm^{-1} and 1,380 cm^{-1}. The atoms directly connected to aliphatic groups could result in a significant change in the standard frequency due to their relatively high electronegativity; in particular, neighboring atoms would move to higher frequencies.

TABLE 3
BET surface area and Langmuir surface area of the catalysts in different temperature.

Sample	BET Surface Area ($m^2\ g^{-1}$)	Langmuir Surface Area ($m^2\ g^{-1}$)
K-OMS-2(423K) / Al_2O_3	270.78	339.24
K-OMS-2(523K) / Al_2O_3	279.78	378.55
K-OMS-2(623K) / Al_2O_3	265.43	360.49

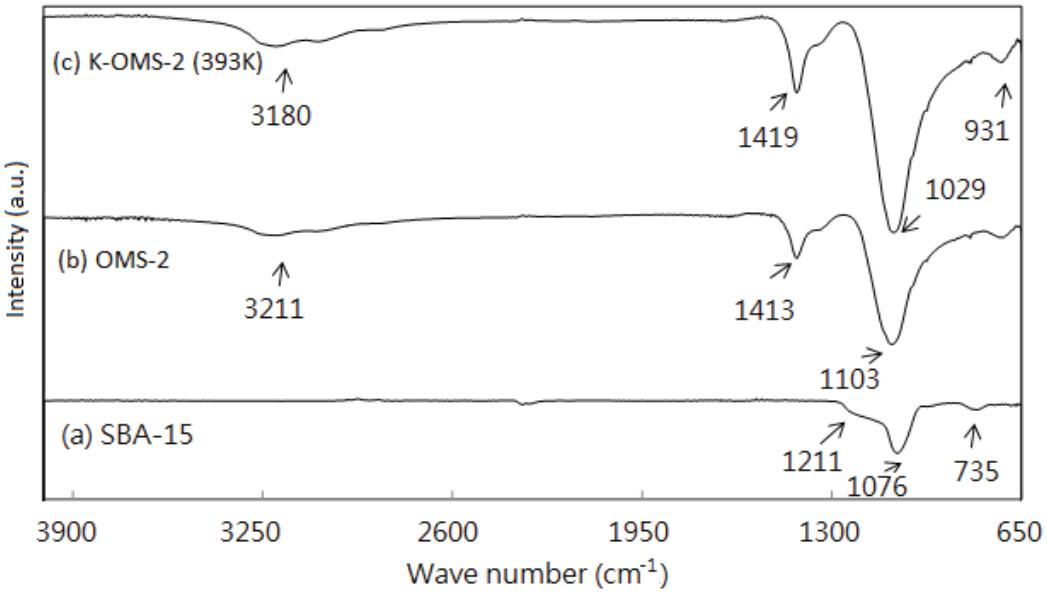

FIGURE 3
FTIR spectra of (a) SBA-15, (b) OMS-2, and (c) K-OMS-2 (393K).

X-ray photoelectron spectroscopy (XPS). The chemical composition and structure of the catalyst could be known by XPS analysis, so the complete pattern and single pattern of the catalyst could be obtained. The full pattern in Figure S3 showed that the peaks of Si and O were found in SBA-15. The complete pattern in Figure S3 showed the S, C, N, O, and Mn peaks of OMS-2 and K-OMS-2, and K-OMS-2 also contained the K peak in addition to the peaks above. The single pattern in Figure S1(a) showed that the binding energy of Si and O in SBA-15 was found at 110.55 eV and 540.10 eV, which was similar to that (Si peak at 103.4 eV and the O peak at 532.9 eV) in the literature [23], proving that the SBA-15 used in this study exhibited the structure of SiO_2. (b) indicated that the binding energy of $Mn2p^{2/3}$ was at 641.9 eV and 642.5 eV, namely, Mn^{3+} and Mn^{4+}, which was the same as the peaks of Mn^{3+}(641 eV) and Mn^{4+} (642 eV) proposed in the literature [24]. (e) S2p showed that S was at 168.65 eV, while the metal sulfate was 166.5–169 eV, which may be the bond of Mn-S or Mn-K. The pattern in Figure S3 N1s showed that N was at 401.5 eV. The K2p peak values of (f) were found at 292.8 and 295.65 eV, similar to 291.5 and 294.5 eV proposed in the literature [25]. It could be inferred that OMS-2 and K-OMS-2 in this study contained S, C, N, O, and Mn; K-OMS-2 also contained the K element, which was consistent with that in the literature.

Thermo-photo-degradation test. First-stage photodegradation. Based on the results of the characteristics analysis of micro/nanorod K-OMS-2 and OMS-2, K-OMS-2 was selected as the photodegradation catalyst, which was applied on the surface of Al_2O_3 and then placed in a first-stage photodegradation system. The experimental parameters include, light source type, and catalyst calcination temperature, based on which the optimal photodegradation operating parameters were selected one by one.

Degradation reactions at different calcination temperatures and light sources. To explore the degradation effect of catalyst at different calcination temperatures, the calcination temperature was kept at 423 K, 523 K, and 623 K for one hour. The results are shown in Figure S4. When SV was 633 hr^{-1}, the optimal calcination temperature was measured to be 523 K by using UVLED as the light source, with an average of 95.56% and average values of 86.69 and 92.75% at 423 K and 523 K, respectively. The results indicated that the catalytic effect of the K-OMS-2/Al_2O_3 catalyst would increase by 1.7% after being calcined at 523 K. Compared with the results of UV-vis analysis, the increase of the absorption value of K-OMS-2/Al_2O_3 catalyst in the UV zone at this calcination temperature was consistent, so that the degradation effect increased. The degradation efficiency at different SVs was shown in Figures S5. First, the space velocity (SV) was taken as the parameter variable, and the calculation formula for SV was SV = reactant flow (L/min)/catalyst reactor volume (L). The SV of the incoming gas is maintained at 1.06 L/min, and the SV is changed based on the amount of K-OMS-2/Al_2O_3 catalyst. When SV was 633, 1,030, and 2,026 hr^{-1}, the catalyst K-OMS-2/Al_2O_3 was illuminated with the light source WLED. When SV was 2,026 hr^{-1}, the toluene removal rate (η) was between 45.29% and 54.90%. Compared with other spatial velocities, the degradation effect was low, and the efficiency tended to decrease gradually. When SV was 1,030 hr^{-1}, the removal rate ranged from 80.31% to 87%, and then tended to decrease slightly. When SV was 633 hr^{-1}, the optimal removal efficiency could be maintained between 87.00% and 94.02%. Therefore, the optimal SV selected at this stage was found when SV is 633 hr^{-1}.

In view of the trend that traditional lamp lighting would be replaced by LED light sources in the future, three kinds of LED lamps—WLED, UVLED, and BLED—were selected as light sources, and the degradation reaction of K-OMS-2/ Al_2O_3 catalyst under different LED light sources was explored. In addition, the residual value of toluene that may be attached to the inner pipe wall of the equipment was obtained by taking glass beads as the background value. Figure 4 shows that when SV was 633 hr^{-1}, the removal efficiency is UVLED >WLED> BLED, and the optimal light source was UVLED, with an average of 93.86%. The average values of WLED and BLED were approximately 80.34% and 79.78%. In the absence of light source, the residual toluene in the inner pipe wall of the equipment was found to be below 5% by taking glass beads as the background value, implying that the removal rate was lower than 5% (blank). By comparing the photocatalytic toluene with three light sources in the absence of catalyst, the removal efficiency of the K-OMS-2 catalyst under the UVLED light source was found to be favorable, with the average removal rates of WLED being 3.97%, BLED being 5.05%, and UVLED being 8.68%. The optimal absorption value of the K-OMS-2 catalyst was approximately 350–500 nm, and the UV wavelength was approximately 380–390 nm. This proved that the optimal absorption wavelength exhibited an obvious influence on the removal rate, and the average photocatalytic efficiency without catalyst was lower than 10%.

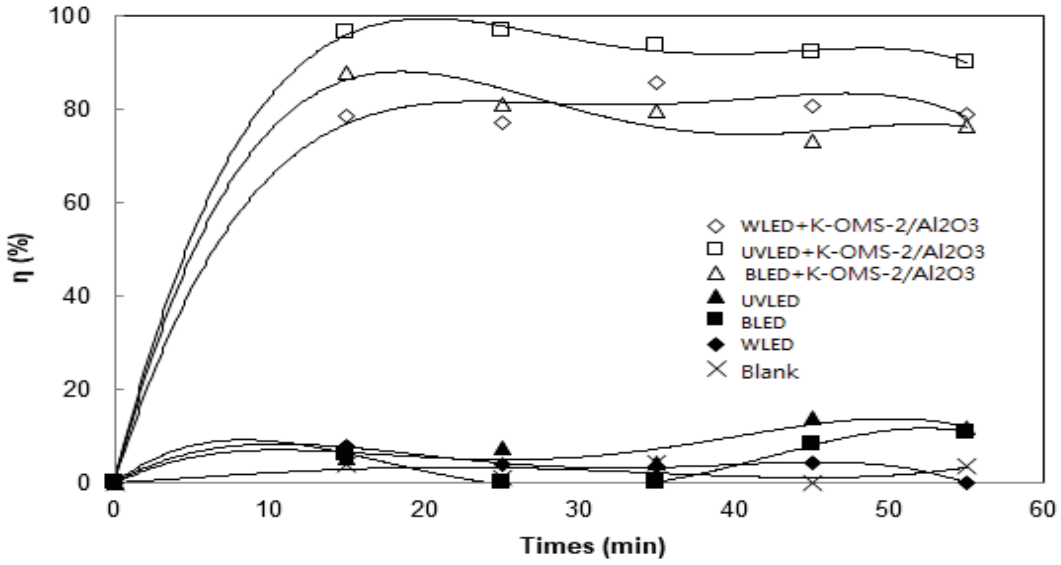

FIGURE 4
Photodegradation removal efficiency of toluene using K-OMS-2/ Al_2O_3 catalyst at space velocities of 633 hr^{-1}under different LED.

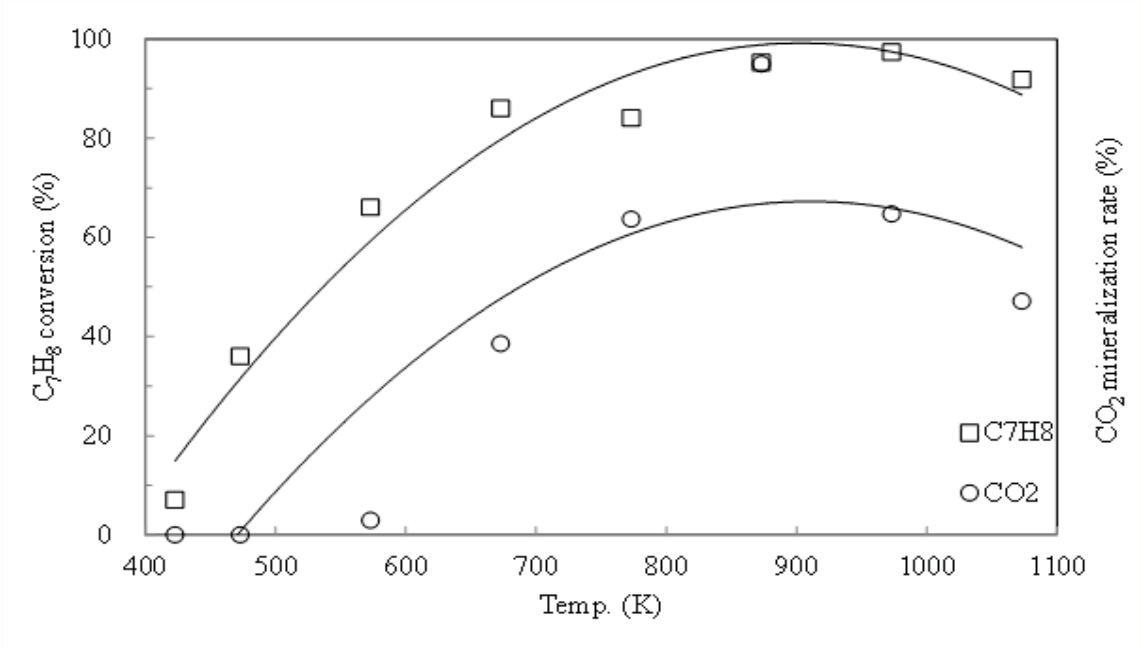

FIGURE 5
Photo-thermo degradation removal efficiency of toluene and CO_2 mineralization rate using K-OMS-2/Al_2O_3 catalyst at different temperature

Analysis of gas product of second-stage thermo-photo-degradation. Under the optimal parameters of photodegradation (K-OMS-2/ Al_2O_3 was calcined at 523 K and placed in a condition of SV of 633 hr^{-1}, and UVLED was used), second-stage thermo-photo co-catalysis was used to enhance the effect of the mineralization rate of CO_2 and to explore the mineralization rate of toluene–CO_2 conversion due to thermocatalysis under different temperatures of the reactor heat source. By comparing the removal rate of toluene by photodegradation, the optimal operating parameters of thermo-photo-degradation were obtained for toluene removal and complete conversion to CO_2. The formula for CO_2 mineralization rate was CO_2 conversion rate (%) = moles of CO_2 consumed / moles of CO_2 introduced x100%.

Thermo-photo-degradation characteristics under different thermo-catalysis conditions. When the K-OMS-2/Al_2O_3 catalyst was placed in the first-stage (heating box) and the second-stage (lighting box) of the reactor and heated at 423, 473, 573, 673, 773, 873, 973, and 1,073 K, respectively, and

illuminated by a UVLED light source, the toluene removal rate was found gradually increase from 7% to 97.36% (Figure 5) at 423–973 K, and decrease to 91.81% at 1,073 K. When the heating temperature reaches 573 K, the catalyst could be used to remove toluene and convert CO_2 to coexistence. At 573–873 K, the conversion rate of CO_2 gradually increased from 2.93% to 95%. When the temperature was higher than 873 K, the conversion rate of CO_2 began to decrease to 47.13%, but the toluene removal rate was still as high as 91.81%. The possible reason was that toluene was converted into CO_2 after being heated. However, CO_2 was reduced to CO and C at a temperature higher than 973 K, resulting in a decrease in the conversion rate of CO_2. Under this second-stage thermo-photo-degradation, the removal rate of toluene was even lower than that of the first-stage photodegradation at some temperatures due to the reverse desorption of toluene attached to the catalyst, which showed that low temperatures would lead to a low toluene removal rate and a low mineralization rate.

In-depth discussion on thermo-photo-degradation. Table S3 summarizes the results of all the tests, including the, calcination temperature, heating temperature, initial concentration, residual concentration, C_7H_8 removal rate, and CO_2 mineralization rate. the UVLED light source could make the catalyst exert a favorable photodegradation effect, and the removal rate of C_7H_8 reached 93.86%. After being illuminated by UV light, electrons of the catalyst were excited from VB to CB, forming electron–hole pairs, and photons were collected on the catalyst surface to generate OH; at the same time, electrons can form O_2 by adsorbing oxygen molecules, and OH could also be generated by $O_2^- + H^+ \rightarrow HOO\cdot$ and $2HOO\cdot \hookrightarrow H_2O_2 + O_2$, so that toluene could be oxidized by OH [20]. The optimal calcination temperature of the catalyst was 523 K, at which the removal rate of C_7H_8 increased to 95.01%, which may be due to the fact that the relatively low calcination temperature (423 K) was insufficient to increase the activity, while the relatively high calcination temperature (623 K) would increase the crystallinity of the catalyst, resulting in a decrease in the total oxidation activity [26].

Based on the aforementioned optimal photodegradation conditions for the removal rate of C_7H_8, and taking different temperatures of heat sources as parameters, the optimal mineralization rate of CO_2 was explored to further obtain the thermal-photodegradation conditions. It was known that when the optimal heat source temperature was 873 K, the mineralization rate of CO_2 reached 95% and the removal rate of C_7H_8 also reached 95.25%. The removal rate of C_7H_8 at 423–773 K under the thermo-photo-degradation condition was lower than that of photodegradation, based on which it was speculated that the pressure in the reaction tube and the SV of gas molecules increased with the increase in temperature, while the optimal temperature range for the pyrolysis of aromatic hydrocarbons was between 773 K and 1473 K [27]. In the case of incomplete pyrolysis, a large amount of toluene gas was forced into the photolysis reactor, resulting in short-term contact between toluene and the catalyst and a limited photodegradation effect. In this study, the relatively higher heat source temperatures, such as 973 K and 1073 K, made the mineralization rate of CO_2 decrease; because toluene decomposition was an endothermic reaction, toluene could generate other gaseous products at relatively high temperatures [28-29]. The reaction of $C_7H_8+9O_2 \rightarrow 7CO_2+4H_2O$ was formed by heating toluene; however, CO_2 may be reduced to CO and C at a relatively high temperature, resulting in a decrease in the CO_2 conversion rate. Based on the research results, this study concluded that the optimal thermo-photo-degradation conditions were SV = 633 hr^{-1}, UVLED light source, and K-OMS-2/Al_2O_3 catalyst. When the catalyst was calcined at 523 K and then reacted in a heat source condition of 873 K, the mineralization rate of CO_2 could reach 95%, and the removal rate of C_7H_8 could reach 95.25% of the thermo-photo-degradation effect.

Analysis of temperatures in the thermal image of thermo-photo-degradation system. This section explores the optimal catalyst conditions of K-OMS-2/Al_2O_3 under calcination temperatures of 523 K and illumination with UVLED at heat source temperatures of 673 K and 873 K to understand whether the temperature change of the catalyst in the later stage was related to the thermal influence of light. The temperature of the catalyst after being calcined at 523 K was 307.71 K, and the standard deviation from the temperature of the catalyst that was not calcined was 1.11, indicating that no increase in heat energy absorbed from the light source was found due to the calcination of the catalyst. The temperatures of the heat sources were 673 K and 873 K, and the average temperatures were 309.34 K and 312.22 K. The temperature of the catalyst in the latter stage increased by 3.03 K when heated at 673 K and illuminated by UVLED. The temperature of the catalyst in the latter stage increased by 6.91 K when heated at 873 K. The thermal image distribution of the catalyst is shown in Figure 6. The results showed that the temperature of the catalyst was relatively low and displayed a green image when it was not illuminated. After being illuminated, it gradually turned into a yellow-red image.

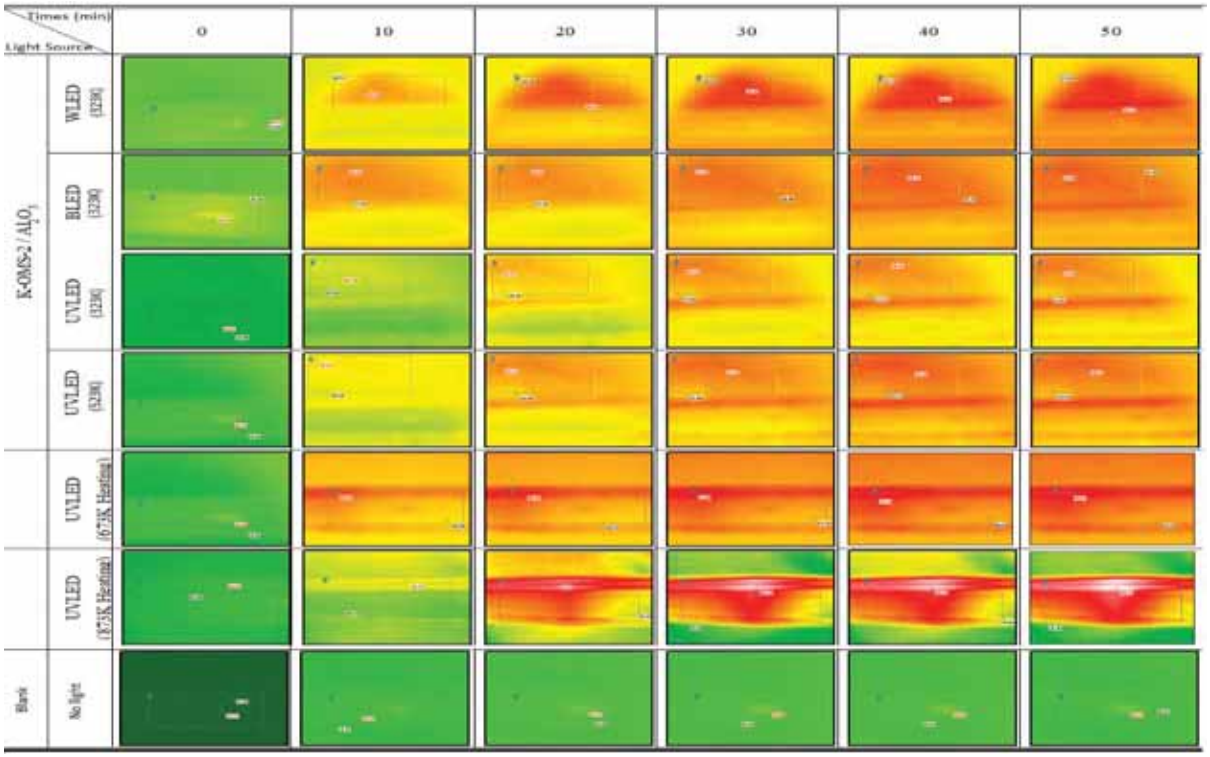

FIGURE 6
Thermal images of catalysts in different source light.

CONCLUSION

In this study, the octahedral manganese ore material of potassium ions (K-OMS-2) was successfully prepared. Additionally, its pollutant removal capacity was studied in the second-stage thermal photocatalytic reaction system using toluene as the target pollutant. Relevant parameters included SV, light source, catalyst calcination temperature, and catalyst reaction temperature. The study showed that the optimal operating conditions of the catalyst were a calcination temperature of 523 K (K-OMS-2/Al_2O_3) and illumination with UVLED illumination at heat source temperatures of 673 K and 873 K. Furthermore, the calcination temperature of 523 K can increase the value of η to 95.56% at the same conditions. However, the CO_2 conversion is not obvious in the photodegradation reaction due to the not complete degradation. In the photo- and thermos-degradation reactor, the situation can be improved and the maximum η of 95.25% and CO_2 conversion of 95% can reach at 873K with UVLED. The result proves that the combined reactor for photo- and thermos-degradation can work in both industrial or domestic application in air pollution control.

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Author Contributions.C.-H. L. and J.-L. S. conceived and designed the experiments, J.-L.S. wrote the paper; Y.-J. Lu and Y. L. performed the experiments and instrument, J.-L. S., M.-H. Y, Y.-H, C. and H.-T. H. designed the process. J.-L. S. and C.-Y. C supervision. All authors have read and agreed to the published version of the manuscript.

Supplementary Materials. The following are available online, Figure S1. XRD patterns, Figure S2. UV/Vis absorption spectra, Figure S3. XPS spectra, Figure S4. Photodegradation removal efficiency of toluene using K-OMS-2/Al_2O_3 catalyst at various calcination temperature under UVLED, Figure S5. Photodegradation removal efficiency of toluene using K-OMS-2/Al_2O_3 catalyst at various space velocities under WLED, Table S1. Illumination of light sources from the top view, Table S2. Illumination of light sources from the bottom view, Table S3 List of all experimental conditions.

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MONITORING SUMMER CHLLOROPHYLIAN ACTIVITY, ALGEA BLOOMS AND SUSPENDED MATTER USING SENTINEL-3 DATA IN GOOGLE EARTH ENGINE IN THE MEDITERANEAN COAST (WESTERN ALGERIA)

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ABSTRACT

The chlorophyll monitoring assesses the abundance of algea blooms in the water. The rate of chlorophyll activity and suspended matter on the coast of Oran (western Algeria) was monitored from physico-chemical analyses of 300 water samples taken during the four-year summer season: 2018, 2019, 2020 and 2021. Remote sensing data available on the Google Earth Engine platform with Sentinel-3 images generated during the same sampling period were also used. The mapping of the variation of the parameters of chlorophyll activity and suspended matter shows the persistence of a gradient parallel to the coast and this takes a center-north direction with the strongest trends. While the eastern zone shows a weakness of activity. This decrease extends, showing a greater transition zone. These results show the appearance of summer algal fluorescences from south to north, which may indicate an expansion of eutrophication in correlation with environmental changes and anthropogenic activities, This allows to highlight the general trends of the sea throughout the year, while exhausting large quantities of oxygen in the water, which increases the pollution of the coastline of Oran.

KEYWORDS:
Monitoring, Chlorophyll, suspended matter, Algae Blooms, Summer, Google Earth Engine, Coastline.

INTRODUCTION

The disruption of chlorophyll activity and accumulation of suspended matter on the Oran coastline in western Algeria are mainly due to water pollution [1, 2]. The reasons are the economic development on the coastal environment which is characterized by high urbanization and various activities [3, 4]. Knowing; the sea contains many species of flora and fauna [5, 6]. It is important that the physico-chemical quality of these waters be continually assessed to ensure that the ecological balance is maintained [7, 8]. In particular, excessive algal abundance is an important indicator of an imbalance [9, 10, 11]. Thus, detection of rate of chlorophyll activity is important for the description of its abundance [14, 15]. This estimate makes it possible to evaluate the marine ecosystem and useful for the detection of cyanobacteria [16, 17]. The cyanobacteria are commonly and naturally occurring in many aquatic systems around the world [18, 19]. They can massively kill fish, contaminate seafood with toxins and negatively alter the ecosystem [20, 21]. If the amount of nutrients in the water is too high, phytoplankton spreads more quickly. The chlorophyll concentrations thus increase [22, 23]. Remote sensing techniques offer significant advantages over in situ monitoring, depending on temporal and spatial coverage [24, 25]. As chlorophyll is a photosynthetic pigment, it causes distinctive changes in water color by absorbing and dispersing sunlight [26, 27]. The chlorophyll level can be estimated from spectral reflection data captured remotely and by linking the reflected light to specific wavelengths at its concentration [28, 29]. The aim of this research is to propose the use of radiometric data for the estimation of chlorophyll activity level, algal proliferation and suspended matter, see their performance using Sentinel-3 images on the Google Earth engine platform. In contrast, in situ estimates of the concentration of chemical components in water such as ammonium, nitrate, nitrite and phosphorus were made at 300 samples points on the Oran coastline.

MATERIALS AND METHODS

Description of the study area. The Algerian coastline spans over 1200 km from Marsat Ben-Mhidi (35° 11’ 38’’ N and 01° 51’ 37’’ W) to El-Kala (36° 53’ 44’’ N and 8° 26’ 36’’ E). The province of Oran is a port city in the Mediterranean, northwest of Algeria. It is located 432 km west of the capital Algiers, its population is 1700000 inhabitants and its area is 2121 km². It is bordered by provinces:

FIGURE 1
Location of study area. The proposed map shows the province of Oran. It is bordered to the north by the Mediterranean Sea, it is a port city of the Mediterranean, and its area is 2,121 km².

to the east by Mostaganem, to the south-east by Mascara, to the south-west by Sidi-Bel-Abbes and to the west by Ain Temouchent. The town of Oran is located at the bottom of a bay open to the north and directly overlooked to the west by the 420 m high Aïdour mountain (Murdjajo) and the plateau of Moulay Abdelkader al-Jilani. The province of Oran is a very important industrial hub [30, 31]. In sedimentological point, the northern end of the Mediterranean of the African continent is extended at sea by a small underwater border; more or less, it ensures the transition with the great depths of Algeria Balearic Basin: the North African continental or pre-continental margin and the North African continental margin. The continental shelf is flat with a very low slope (0 to 46°), extending between the zero level and the first inflection of the bottom between (90 m and 120 m) [32, 33]. The sedimentary cover of this plateau is varied in nature, starting with the limestone, which is the main constituent of the sediment; it forms a carpet covering the entire continental shelf, compared to the cal-sludge. They cover a large part of the Gulf of Oran, are very rich in rock debris and fresh, transparent shells, while silky clay mud represents a small percentage on the edges of this golf course [34, 35]. In the hydrological point, the Algerian basin is subject to the direct influence of the very turbulent Atlantic current which is related to the proximity of the Strait of Gibraltar and is of great importance in the Oran coastline. The climate is of the Mediterranean type, particularly warm in summer and mild in winter, these conditions are due to the alternation of cool and wet breezes and warm and dry breezes [36, 37]. The rainfall varies from year to year, is one of the lowest in northern Algeria, varies between 350 mm and 400 mm and cannot exceed 200 mm to 250 mm, in some dry years [38, 39]. The coastal waters of Oran are exposed to various forms of pollution whose origin remains: industries, uncontrolled urban expansion, tourist concentration and economic and social development cause the degradation of natural environments [40, 41].

Physicochemical analyses. Three hundred monitoring points on the Oran coast were chosen for the physico-chemical analysis of the water. These points were located near urban areas, commercial ports and other coastal human activities (Figure 2). Samples were collected in the summer of 2018, 2019, 2020 and 2021. The nutrients considered are nitrite, nitrate, ammonium, total phosphorus; were analyzed in the laboratory using the standard colorimetric method [42, 43]. As a reminder; ammonium Ammonia exists in water in two main forms: NH_3^+ and NH_4^+. At its usual pH, ammonium (NH_4^+) remains the most dominant form. Ammonium assay is

performed using the Koroleff (1969) method. This assay determines all the ammo-niacal nitrogen. Determination of nitrate (NO_3) after reducing nitrate ions to nitrite ions, the method applied is the Bendschneider & Robinson (1952) method. The determination of nitrites (NO_2) is made using the Bendschneider & Robinson (1952) method. Nitrite ions form a diazoic complex with sulphanilamide in an acid medium. Phosphates are all dissolved forms of the orthophosphate ion (PO^3_4). Their assay is carried out according to the method of Murphy & Riley (1962). This test makes it possible to determine the orthophosphate ions dissolved in seawater. Phosphate ions react with ammonium molybdate, in the presence of antimony, to form a complex that is reduced by adding ascorbic acid. In our study; all water parameter concentrations are expressed in mg/l.

Sentinel-3 data processing. In this study, the use of remote sensing as a means of surveillance is an inevitable alternative, thanks in particular to the availability of various sensors orbiting the earth; these on-board instruments provide access to several information on marine ecosystems [42, 43]. In this sense, we have chosen to exploit the data provided by the sentinel-3 sensors OLCI (ocean and earth color); From the COPERNICUS program of the European Space Agency. Sentinel-3 is a constellation of satellites orbiting the earth with the aim of acquiring data on the topography of the seas and oceans, the temperature of the seas and continents and their colors, Due to the high rotation frequency and availability of data, we have chosen to use data that is made available to users online for free [44, 45].

However, given the large amount of data required for our research and to avoid the costly procedures of downloading images one by one, we opted for the use of cloud computing platforms like Google Earth Engine (GEE) for acquisition speed, processing of different data sources [46, 47]. Google Earth Engine is a cloud computing platform that has become essential for the diversity of data available to users, but also for the ease of use, especially in downloading and processing time series of satellite images. The sentinel-3 data available on the platform are the sentinel-3B OLCI EFR data, including radiance values [48, 49]. The Ocean and Land Color Instrument (OLCI) Full Resolution Earth Observation (EFR) dataset contains radiances from the top of the atmosphere in 21 spectral bands with center wavelengths between 0.4 µm and 1.02 µm at 300 m spatial resolution with global coverage every 2 days [50, 51]. Knowing that it is not possible to generate geophysical parameters from radiance data, and faced with the impossibility of performing atmospheric corrections directly on the GEE platform, we chose to write a script allowing to download the scenes corresponding to the period (put the sampling period) which corresponds to the sampling campaign of the study site in summer 2020. Moreover, knowing that the sentinel-3 images used have 21 spectral bands allowing different information, we have chosen to download only the spectral bands corresponding to the chlorophyll activity and the organic matter in suspension in the water corresponding only to the study site (Table n°1). The atmospheric correction was carried out according to an appropriate algorithm. The downloaded and listed data was processed to obtain the reflectance values using a script written in python under the SNAPPY module, a utility developed by the European Space Agency for processing sentinel data [52, 53]. About linking and matching data to sample points; the reflectance-corrected images are subjected to a supervised classification of the maximum likelihood by pressing on regions of interest which correspond to each monitoring point with the software (Environment for Visualizing Images ENVI, version 5.1). It is one of the most popular classification methods in remote sensing, in which a pixel with the maximum likelihood is classified into the corresponding class. The likelihood is defined as the posterior probability of a pixel belonging to class k. The maximum likelihood method has an advantage from the perspective of probability theory, but attention should be paid to the following. Sufficient ground truth data must be sampled to allow estimation of the population mean vector and variance-covariance matrix. The inverse matrix of the variance-covariance matrix becomes unstable in case there is a very high correlation between two bands or the ground truth data is very homogeneous. In such cases, the number of bands should be reduced by principal component analysis. When the population distribution does not follow the normal distribution, the maximum likelihood method cannot be applied [54, 55]. The classified images are integrated into a geographic information system for the extraction of the values corresponding to each sampling point, with the aim of finding a correspondence (correlation) between the measurements by the onboard instruments and the various parameters obtained in the laboratory after sample analysis.

Statistical and Spatial Analysis. We studied the correlations between the averages and the variances of all the physi-co-chemical parameters and the levels of the reflectance which indicates the spectral response rate of the chlorophyll activity and the suspended matter of the water obtained. The maps finally retained for the chlorophyll activity parameters are: the chlorophyll reference (Chl-min), max chlorophyll absorption (Chl-max), chlorophyll vegetation (Chl-Veg). For bloom algae area, the parameters are: chlorophyll fluorescence red border (chl-red), chlorophyll fluorescence red border (Modis-Chl). For suspended matter, the parameters are: yellow and detrital pigment substance (yellow-sub), sediment loading rate (sedi-lod), turbidity (Sedi-Turbi). The main real threat to the validity of significance tests arises when the means of the variables

by group are correlated with the variances (or standard deviations). Intuitively, if there is high scatter in a group with particularly high means for a variable, those high means will not be reliable. However, tests of overall significance are based on pooled variances, that is, the average variance of all groups. Therefore, significance tests for the relatively larger means (with large variances) are based on the relatively smaller pooled variances, inducing erroneous statistical significance. In practice, this situation can arise when a study group has a few atypical points, strongly impacting the averages, and contributing to increasing the dispersion. To counter this problem, carefully examine descriptive statistics, especially correlations between means and standard deviations or variances. Principal component analysis concerns the calculation of the principal components of the associated statistics. We performed this analysis on images in the pre-processing phase using ENVI 5.1 software to visually classify variables and observations. In fact, we often analyze the scalar product of these matrices, to clarify the interpretation of the results. The spatial representation of the results obtained by the interpolation method is also retained to study the distribution and the limit in space of the physico-chemical elements of water. Spatial analyzes were performed using ArcGis 10.2 software. Finally; we have opted for the superposition of the maps of chlorophyll activity and turbidity with those of the physico-chemical elements to visualize the zones vulnerable to pollution in the Oran coastline.

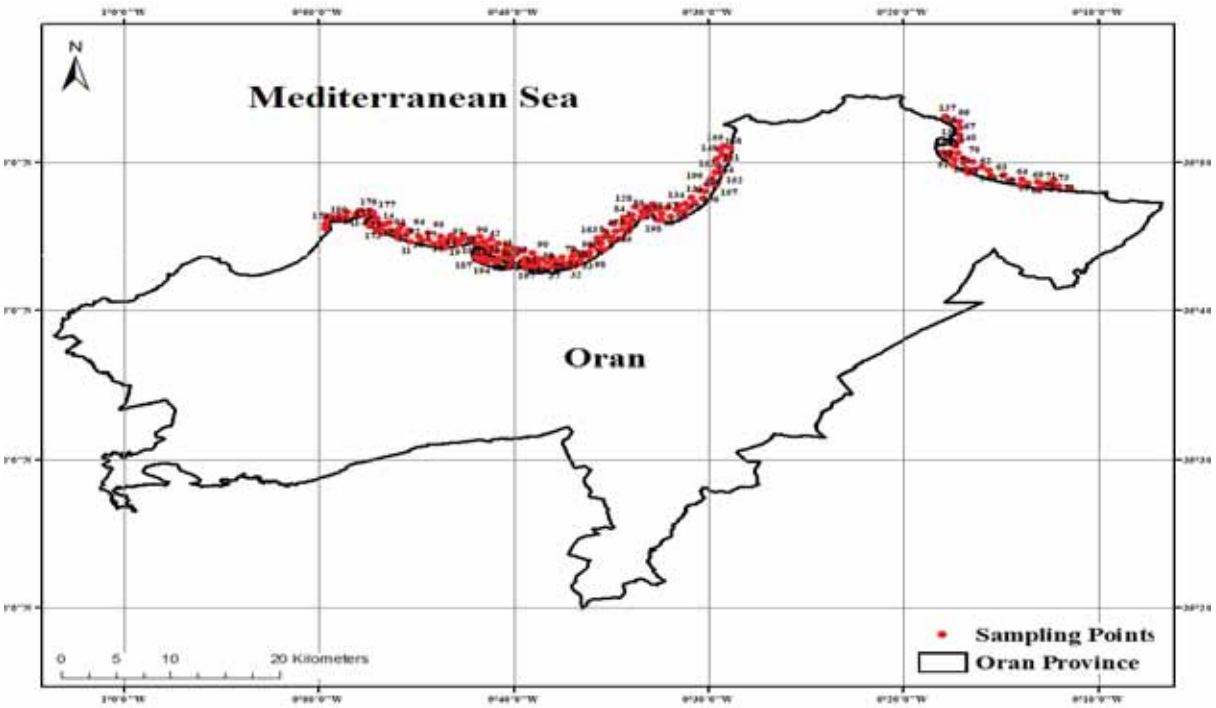

FIGURE 2
Location of samples points. The 300 monitoring sites are located to urban areas, commercial ports and other coastal human activities in Oran coastline. In the table 1 of supplementary materials; the geographical coordinates of each point were mentioned.

TABLE 1
Sentinnel-3 images downloaded from Google Earth Engine summer 2020.

N	Name	Units	Scale	Wavelength	Description
1	Oa02_radiance	Wm^-2sr^-1 µm^-1	0.0133873	412.5nm/10nm	Yellow substance and detrital pigments
2	Oa03_radiance	Wm^-2 sr^-1 µm^-1	0.0121481	442.5nm/10nm	Chl absorption max, vegetation
3	Oa04_radiance	Wm^-2 sr^-1 µm^-1	0.0115198	490nm/10nm	High Chl, other pigments
4	Oa05_radiance	Wm^-2 sr^-1 µm^-1	0.0100953	510nm/10nm	Chl, sediment, turbidity, redtide
5	Oa06_radiance	Wm^-2 sr^-1 µm^-1	0.0123538	560nm/10nm	Chlorophyll reference (Chl minimum)
6	Oa07_radiance	W m^-2 sr^-1 µm^-1	0.00879161	620nm/10nm	Sediment loading
7	Oa08_radiance	W m^-2 sr^-1 µm^-1	0.00876539	665nm/10nm	Chl absorbtion max, sediment
8	Oa10_radiance	W m^-2 sr^-1 µm^-1	0.00773378	681.25nm/7.5nm	Chl fluorescence peak, rededge
9	Oa11_radiance	W m^-2 sr^-1 µm^-1	0.00675523	708.75nm/10nm	Chl fluorescence rededge transition

FIGURE 3
Photos of Oran coastline. (a) tourism; (b) coastal cities; and (c) miscellaneous commercial activities.

TABLE 2.
Results of physico-chemical analysis of 300 monitoring samples points in Oran coastline.

N=300	Nitrite	Nitrate	Ammonium	Phosphorus
Min	0,003	0,004	0,067	0,033
Max	1,567	3,279	6,861	3,876
Moy	0,409	0,756	1,727	0,803
St-dev	0,289	0,496	1,123	0,580

*This table illustrates the values of: maximum, minimum, average and standard deviation of physico-chemical water nutrient.

RESULTS

Physicochemical water nutrient. The main physicochemical analysis results obtained are shown in mg/l the following table 2. Note that the most remarkable concentration values are retained for ammonium (with a minimum of 0.1200 mg/l and a maximum of 6.8600 mg/l) and nitrite (with a minimum of 0.0900 mg/l and a maximum of 1.6230 mg/l). The average value of 1.0510 ± 0.60298mg/l was recorded for nitrates, phosphorus shows significant concentrations with 1.1140 ± 0.54939 mg/l on average.

Spatial Analysis of physicochemical water nutrient. We note that the lowest concentrations of nitrate persist in the parallel band close to the coast, whose values belong to class C1 [0.04 mg/l, 0.36mg/l]. At a distance of a few meters we notice the progressive evolution of the concentrations, let us quote the main classes: C2[0.36 mg/l, 0.58mg/l], C3[0.58 mg/l, 0.80mg/l], C4[0.80 mg /l, 0.99mg/l] and C5[0.99mg/l, 1.19mg/l] (Figure 4). The extreme western zone shows significant concentrations: C6[1.19 mg/l, 1.46mg/l], C7[1.46 mg/l, 2.05mg/l] and C8[2.05 mg/l, 3.04mg/l]. A very remarkable central band in the center of the study perimeter, it is characterized by the presence of high values of nitrite; include classes C6[0.57 mg/l, 0.73mg/l], C7[0.73 mg/l, 0.96mg/l] and C8[0.96mg/l, 1.37mg/l]. This gradient persists towards the north. The rest of the study area marks medium to low values with an increasing gradient towards the north. The presence of the following classes as: C1[0.03 mg/l, 0.14mg/l], C2[0.14 mg/l, 0.25mg/l], C3[0.25mg/l, 0.35mg/l], C4[0.35 mg/l, 0.45mg/l] and C5[0.45mg/l, 0.57mg/l]. The concentrations of

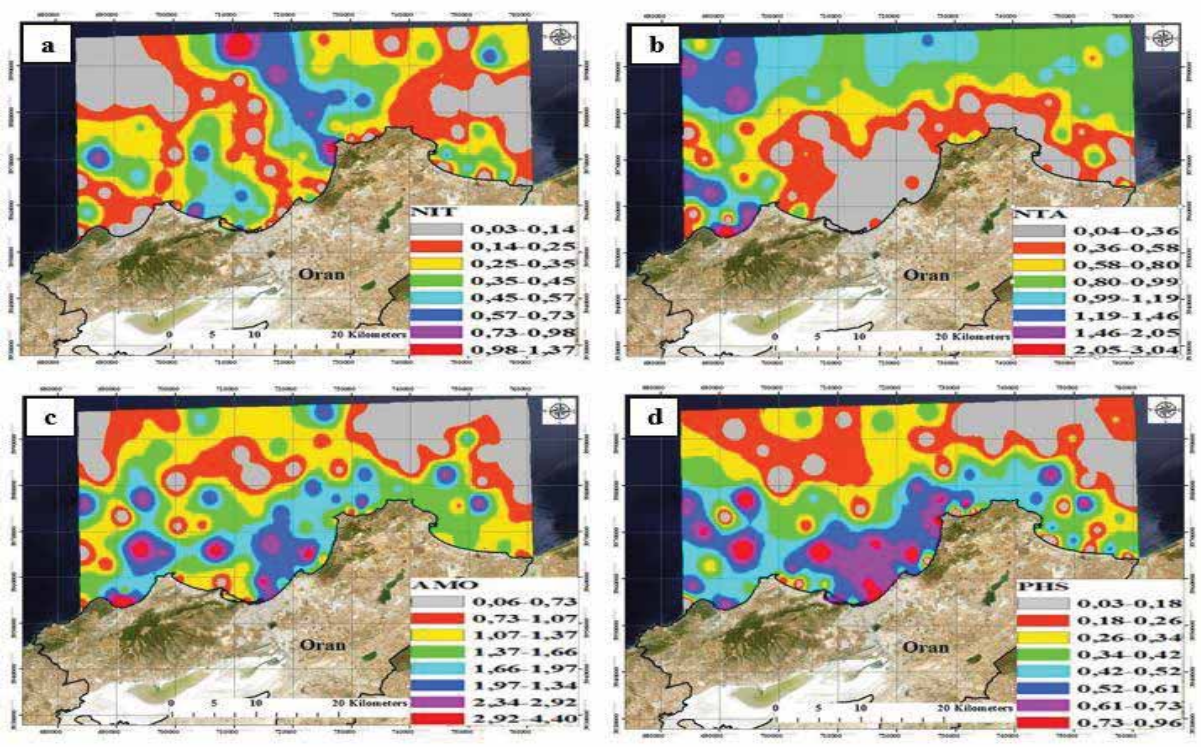

FIGURE 4

Spatial Analysis of physicochemical water. (a) Spatial distribution of nitrate concentration ;(b) Spatial distribution of nitrite concentration; (c) Spatial distribution of ammonium concentration; (c) Spatial distribution of phosphorus concentration.

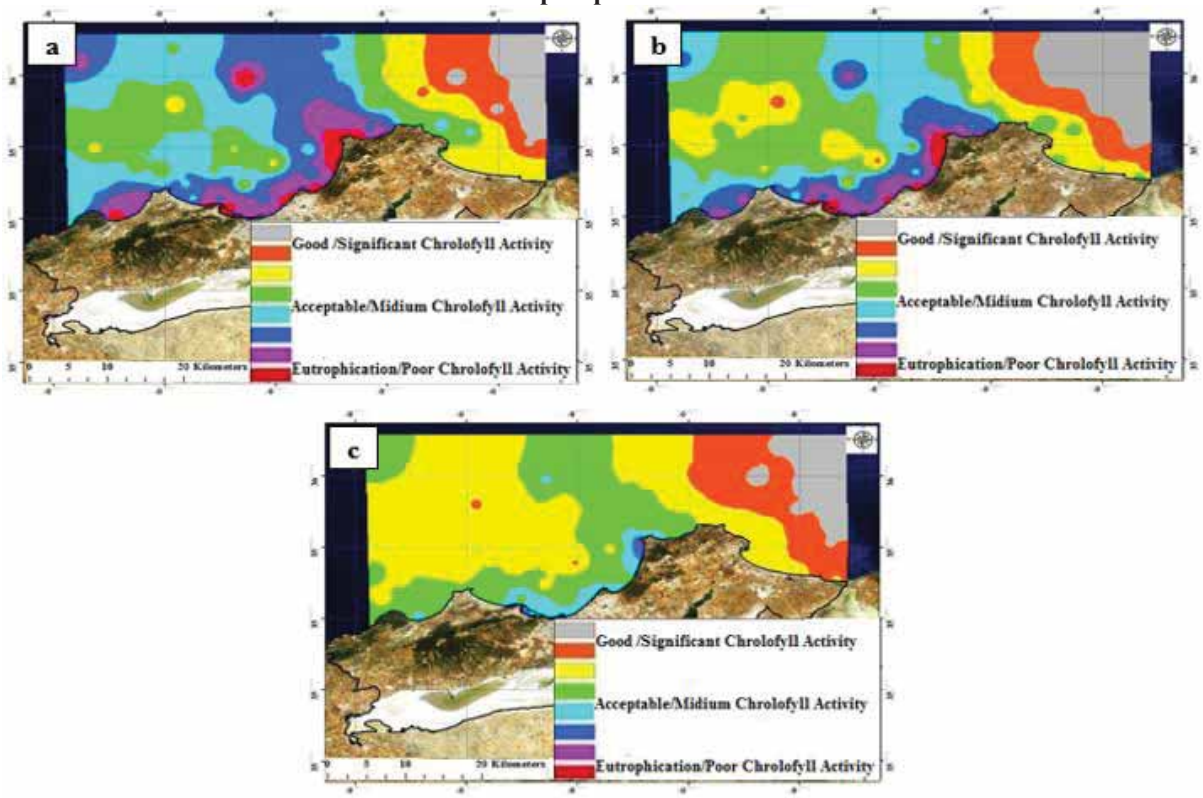

FIGURE 5

Mapping of intensity of activity chlorophyll in Oran coastline.

We distinguish tree classes according to the level of chlorophyll: Good significant activity, acceptable medium activity and eutrophication poor activity. (a) Activity chlorophyll minimum; (b) activity chlorophyll maximum; (c) activity chlorophyll vegetation

phosphorus is very notable, we note that the high values persist in the central and western part. An increasing gradient towards the north is recorded in the eastern part. phosphorus culminates in the following classes: C1[0.03 mg/l, 0.18mg/l], C2[0.18 mg/l, 0.26mg/l], C3[0.26mg/l, 0.34mg/l], C4[0.34mg /l, 0.42mg/l], C5[0.42mg/l, 0.52mg/l], C6[0.52mg/l, 0.61mg/l], C7[0.61mg/l, 0.73mg/l], C8[0.73mg/l, 0.96mg/l]. For ammonium, the concentrations are very diverse throughout the study area. We note the distribution of the poles where the values follow a decreasing gradient and other poles whose concentrations are increasing. Ammonium concentrations range from 0.06mg/l to 4.40mg/l. the classes selected are: C1[0.06 mg/l, 0.73mg/l], C2[0.73 mg/l, 1.07mg/l], C3[1.07mg/l, 1.37mg/l], C4[1.37mg/l, 1.66mg/l], C5[1.66mg/l, 1.97mg/l], C6[1.97mg/l, 2.34mg/l], C7[2.34 mg/l, 2.92mg/l], C8[2.92mg/l, 4.40mg/l]

Mapping of chlorophyll activity parameters. The maximum production of chlorophyll (Chl-max) shows a band parallel to the coastline where urbanization and anthropogenic activities are abundant. The concentrations are very high. The extreme eastern part of the study area shows low chlorophyll activity. The central western part records an average activity (Figure 5).

For the beginning of accumulation of the reference chlorophyll (Chl-min); we observe the persistence of a very visible band near the coast whose activity. Note that a strong activity whose spectral response is very important. The east coast is marked by a weakness of activity with the spreading of this gradient towards the north. The bands Chlorophyll Vegetation (Chl-veg) takes the vertical parallel to the north, distinguishing two zones. The first is located in the eastern part, this gradient containing low activity. The second central zone with important activity (Figure S1 (a);(b);(c);(d)).

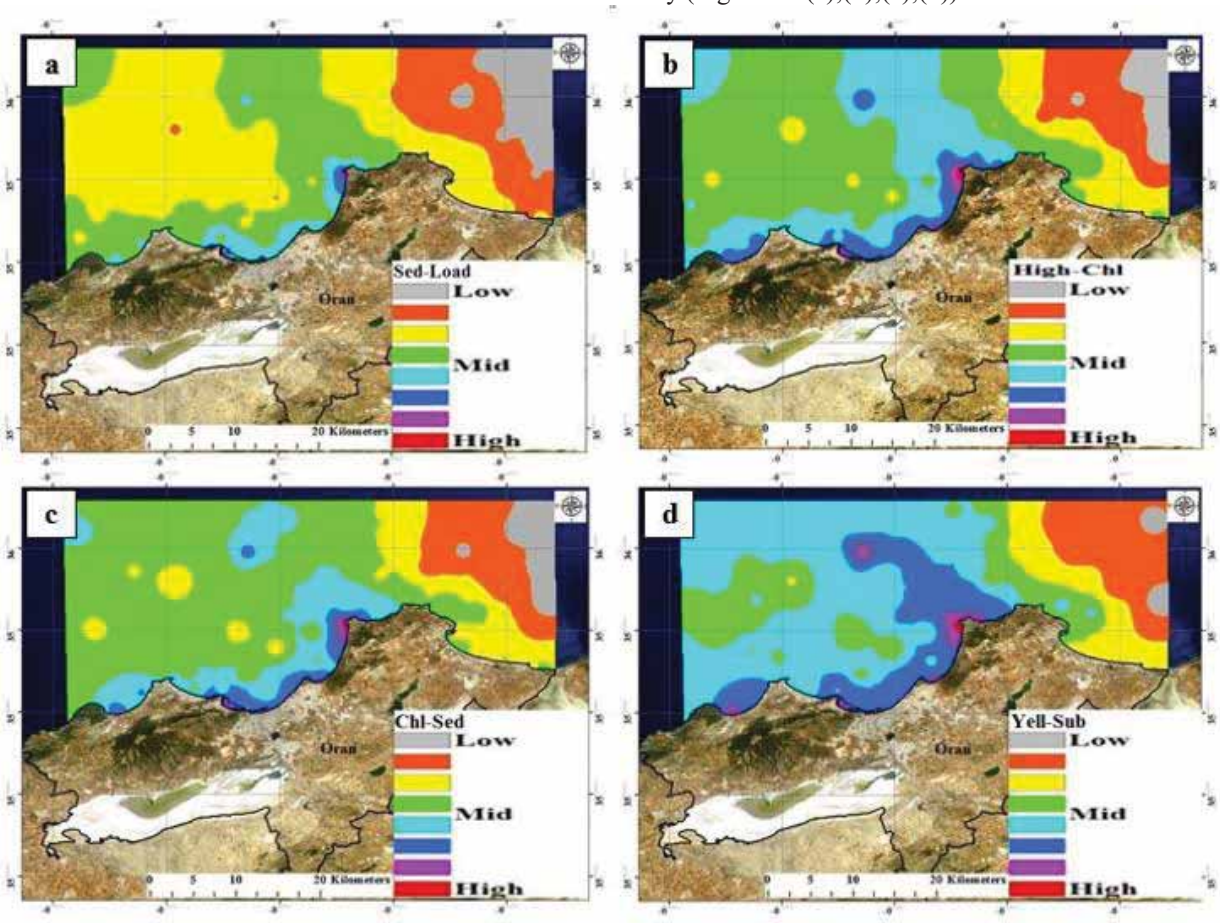

FIGURE 6
Mapping of rate of accumulation of suspended matter in Oran coastline. We distinguish tree classes: high accumulation, medium accumulation and low accumulation. (a) sediment loading; (b) Other pigment of Chlorophyll; (c) Chlorophyll sediment and turbidity; (d) Yellow substance and detrital pigments.

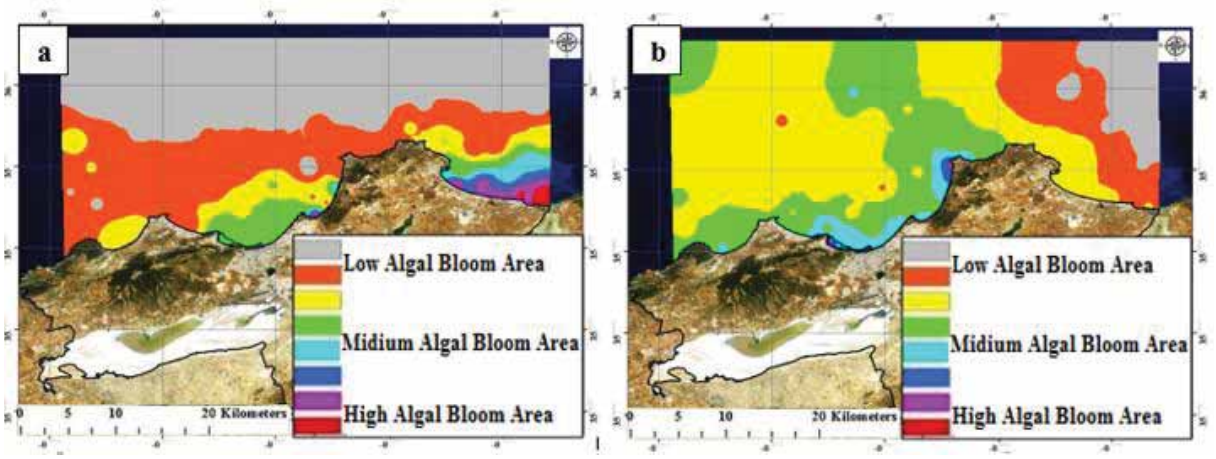

FIGURE 7
Mapping of algea bloom sarea in Oran coastline. We distinguish tree classes: high algea bloomsarea, medium algea blooms area and low algea blooms area. (a) Maximum Chlorophyll fluorescence; (b) Minimum Chlorophyll fluorescence.

Mapping of suspended matter parameters. The sediment loading (sedi-lod) rate is very low in the eastern part and marks the low values of reflectance. We notice the majority of the coastline almost a few kilometers near urban areas records a significant sediment loading rate (Figure 6) For Yellow substance and detrital pigments (yellow-sub) in the eastern part; we observe a vertical gradient crossing green the central zone of the coast with the persistence of low rate. We are heading towards the central and western part; we note the presence of significant concentrations. The Chlorophyll sediment and turbidity (Chl-sed) is significant with remarkable quantities in horizontal parallel of the Oran coast. Two small poles are formed with only one class of high concentrations; one in the center of the study area, and the other in the west. Other pigment of Chlorophyll (High-Chl) shows the same trend as chlorophyll turbidity; whose rate of accumulation of suspended solids is persistent in a gradient from east to north. The central and western part records the highest concentrations (Figure S3 (a);(b);(c);(d), Figure S4 (a);(b);(c);(d)).

Mapping of algae blooms parameters. The band of the minimum of Chlorophyll fluorescence red edge (Modis-Chl) is narrow with the parallel of the coastline of Oran where we can distinguish two poles, the first is located in the extreme eastern part; whose gradient is very important. The central and western part of the coast is marked by the presence of only highest values (Figure 7).

The maximum Chlorophyll fluorescence red edge (chl-red) takes a different path than that of Chlorophyll fluorescence red edge (Modis-Chl). The band takes the vertical parallel to the north, distinguishing two zones. The first is located in the eastern part, this gradient containing the low values. The second central zone and extends to the north only by the medium values. We observe that this gradient resumes its horizontal direction towards the west (Figure S2 (a);(b);(c);(d)).

DISCUSSION

The monitoring of coastal and marine ecosystems is done through the evaluation of the physicochemical nutrient which make it possible to characterize the quality of the water [56, 57], in order to identify the spatial evolution in the understanding of the eutrophication process, the detection of the abundance of algae and more particularly the dynamics of chlorophyll activity. These measurements are influenced by continental and anthropogenic inputs [58, 59] and are important indicators of imbalance for the overall characterization of the aquatic system [56, 59]. This work constitutes the main results obtained for the sampling campaign of the summer of years: 2018, 2019, 2020 and 2021 in the Oran coast, making it possible to characterize the water masses sampled at the monitoring points. The method adopted was physico-chemical analysis of water from three hundred sampling points; this step was completed by the remote sensing method to estimate chlorophyll activity, algal fluorescence and suspended matter. Coastal inputs related to human activities combined with the effects of climate change have multiple consequences attributable to eutrophication phenomena [59, 61]. It was therefore important to simultaneously acquire field data [60, 61]. The estimation of chlorophyll concentrations is one of most scientifically revelant and commonly used applications of remote sensing to aquatic coastal systems because of the complex composition and distribution of optically active constituents [62, 63]. The measurements of fluorescence of phytoplankton are benefiacial for monitoring water quality due to the dynamic nature of such environments and is facilitated by satellite

remote sensing particulary medium resolution sensors such as modis, meris and OLCI [62, 63]. From the results of the physico-chemical analyzes of the sampled waters, we can say that the sea waters of the Oran coast are characterized by: a high concentration of ammonium of 6.86 mg / l maximum, phosphorus total and nitrates miss concentrations range between 0.05mg/l and 3.94mg/l while nitrites are in 1.62 mg/l maximum. Ammonium shows small variations on all the points, the standard deviation is estimated at 1.26. However, relatively low values are observed in summer with more or less significant fluctuations indicating that the concentrations at the coast are greater. The nitrite and nitrate concentrations show significant fluctuations from one point to another. The levels are on average very low in summer, only 0.49 mg/l and 1.05 mg/l. However, exceptionally high concentrations are observed at sites along the coast. The concentrations of the nutrients recorded are reduced compared to the other seasons which coincide with the period of optimal chlorophyll activity. Nitrates and nitrites are naturally occurring ions in the environment [64, 65]. They are the result of nitrification of the ammonium ion is strongly linked to the dynamics of nutrient salts such as nitrates and phosphates [64, 67]. Ammonium is the average seasonal cycle of ammoniacal nitrogen [66, 67]. These elements are required for the assessment of the influence of continental nutrient inputs on possible eutrophication processes in the marine environment [68, 69]. According to the monitoring of nutrient dynamics along the northern coast in Picardy by Nzigou and Lefebvre, 2013; that the maximum concentrations of nitrate and nitrite are observed in the winter period, while the minimum concentrations are recorded for the summer season [59, 70]. The seasonal dynamics of phosphorus are marked by high values in winter and autumn and low values during spring and tend to increase for the summer season and then reach high values in winter [59, 70]. Ammonium concentrations are lower in spring and summer and higher in winter and fall. This dynamic is consistent with nutrient utilization during the productive period [59, 70]. According to Jaquet, 2002; the limiting factor for phytoplankton growth is the presence of nutrients. These can be either of natural origin, such as upwelling zones or cold currents, or of anthropogenic origin: leaching of fertilizers, discharge into the sea of wastewater of various kinds (household, industrial, chemical) [70, 71]. The common temporal dynamics of all the physico-chemical parameters observed during the year follows the classic pattern of a temperate coastal system by the influence of hydrodynamics on coastal water temperatures [72, 73]. The mapping of chlorophyll activity estimates during the summer season shows the persistence of a very visible band near the coast whose activity is very important and high. The chlorophyll activity is slightly different between the sampling points except at the coastal points where slight variations are recorded. This can be explained by the formation of an algal biodiversity along the coast. The variation in chlorophyll concentrations shows a classic dynamic characterized by low activity in winter and autumn followed by high activity in spring and summer [74, 75]. Chlorophyll-a concentration is considered to be an indicator of total autotrophic biomass, as this abundant pigment is present in all plant organisms [72, 74]. According to Field et al. 1998; Siano 2007; ocean phytoplankton are responsible for about 50% of the annual primary production on Earth while they represent only 1% of the global biomass of the oceans [75, 76]. They obtained their primitive plastids from an ancestral cyanobacterium endowed with photosynthetic capacities. According to Reynolds 2006, Phytoplankton dynamics are particularly important in coastal areas, favored by the supply of nutrients from rivers, exchanges with offshore water masses and with sediment in shallow areas [73, 76]. It is closely linked to the variability of water quality, hence the fact that phytoplankton is often used as an indicator of the degradation of water bodies resulting from human activities [76, 77]. Phytoplankton react differently depending on the organisms to spatio-temporal changes in the environment [78, 79], because the physiological and morphological diversity of these organisms reflects the many strategies of life and adaptation to their environment [76, 79]. Pigments are part of the photosynthetic machinery of phytoplankton [77, 78]. They can be directly associated with: the capture of light and its conversion into chemical energy (photosynthetic pigments), the transmission of light energy to the photosystems, or photoprotection by allowing the dissipation of excess energy by fluorescence including the appearance of non-photosynthetic or accessory pigments [80, 81]. The estimation of fluorescence makes it possible to highlight efflorescences in the form of blooms [82, 83]. Our results obtained by fluorescence tau mapping; shows the appearance of significant bloom areas in the summer season in parallel along the coast of Oran. These chlorophyll biomasses are stronger on the monitoring sites, the latter being under the significant influence of the effect of a high concentration of nutrients, favoring significant blooms and greater variability during the productive period. The concentrations of organic matter during the productive period can be explained by the appearance during this same period of certain species responsible for phytoplankton blooms [84, 85]. Blooms are initiated in March to reach their highest peaks in the spring and summer season coinciding with the algea blooms period. A faint bloom also occurs in early fall [86, 87]. The dynamics of suspended solids and consequently the turbidity in the coastal zone play a major role in the productivity of these systems [86, 88]. It is indeed linked to phytoplankton development which predominates during periods of high biological productivity [88, 89]. The

values are higher in summer indicating greater production activity at the sampling sites. The fluctuations that are observed are related to sedimentary dynamics [90, 91]. Studies in estuarine waters have shown that the organic matter in the sediment generally remains very low [92, 93]. High organic matter concentrations also determine mineralization rates by heterotrophic bacteria [94, 95]. According to Brylinsky and Lagadeuc, 1990; The coastal water fringe is clearly influenced by land inputs: salinity is lower there than offshore and turbidity values are higher. Turbidity results from the presence of mineral or organic particles in suspension but also from the high concentration of living organisms [89, 95]. An increase in turbidity is the direct consequence of movements under the action of wind and currents, correlated with the tidal coefficient [92, 94]. The dynamics of suspended solids and consequently that of turbidity in the coastal zone play a major role in the productivity of these systems [93, 95]. Wind also plays a particularly important role in the resuspension, dispersion and regulation of turbidity between the coasts and the open sea [94, 95]. Wind direction has a strong influence on coastal dispersion [90, 95].

CONSLUSION

On the Oran coast and using Sentinel-3 images on the Google Earth engine; a mapping of the chlorophyll activity rate, areas of algal blooms and suspended solids using physico-chemical analyzes at three hundred measurement points was carried out. Our overall results show that the study area is characterized by a classic nutrient dynamics which shows low concentrations in summer describing a production and consumption regime. Dysfunction of chlorophyll activity due to nutrient loading is expected to increase the severity of eutrophication and algea blooms formation. the persistence of an increasing gradient of the algea blooms and the sediments parallel to the coast is closely linked to the increase in the content of nitrite and phosphorus in the water, the further one goes from the coast this gradient becomes decreasing. Remote sensing for estimating chlorophyll and turbidity levels from reflectance observations in Sentinel-3 images from the European Agency seems relevant for this contribution. An understanding of Chlorophyll dynamics is important for ecosystem restoration. In this case to complete this work, a project to assess the dynamics of biodiversity in phytoplankton is being carried out on the same monitoring sites. This would also make it possible to build larger databases containing useful information for a better appreciation of the local ecological context and preservation of the aquatic environment.

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COMPARATIVE TOXIC EFFECTS OF CHEMICAL AND BOTANICAL INSECTICIDES ON THE REPRODUCTION AND OXIDATIVE STRESS PARAMETERS IN THE *Wistar* MALE RAT

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ABSTRACT

Occupational and environmental exposure to pesticides, that induce oxidative damage may impair male fertility. The present study aims to compare the toxic effects of a chemical insecticide (Imidacloprid) against two bio-pesticides (Azadirachtin) and aqueous garlic extract on male fertility and oxidative stress parameters in *Wistar* male rats. Animals were divided into 7 groups, the G1 served as the control group. G2, G3, G4, and G5 were orally fed Azadirachtin and Imidocloropid at 1/30 and 1/15 LD50, respectively. G6 and G7 were treated with 1/30 and 1/15 LD50 garlic aqueous extract for 6 weeks. The results showed a decrease in absolute testis mass in Imidocloropid and Azadirachtin-treated groups, and a significant decrease in testosterone level in Imidacloprid-treated groups compared to the control group. Sperm concentration, motility, viability, velocity, amplitude of lateral head displacement, and beat cross frequency were all significantly lower in Imidacloprid treated groups. In addition, as compared to the control group, these pesticides caused an increase in oxidative stress parameters such as MDA levels and a substantial decrease in GSH and GPx levels. Thus, we suggested that the bio-insecticide Azadirachtin was not strictly safe, and the aqueous garlic extract was less toxic than the two insecticides regarding its negative effects on male reproductive and oxidative stress parameters.

KEYWORDS:
Chemical pesticide, fertility, insecticide, bio-insecticides, oxidative stress, rats

INTRODUCTION

Agricultural productivity, can be adversely affected by numerous pests [1]. To maintain agriculture production at high levels, pesticides have been extensively used, but require careful identification of the factors impacting livestock or crop production so that on-time application using the recommended method can be realized [2]. In many instances, pesticides have been proven successful at reducing pest populations, yet also had several unwanted environmental consequences owing to the negative effects they incur on non-target species [3]. They cause acute poisoning in mammals, as well as long-term health impacts, such as adverse effects on reproduction [4].

Among neonicotinoids, Imidacloprid have being intensively used over the years, representing a major class of highly potent insecticides sprayed for crop protection against piercing-sucking insects [5], yet although having adverse effects on non-target species, and could affect reproductive function [6], [7], reduce sperm count, motility, and viability [8], and cause oxidative effects by reducing testis GSH, and GPx [9]. Subchronic exposure to Imidacloprid can cause sperm abnormal velocity, and can also reduce the testosterone levels in male rats [10]. All of these alterations can lead to infertility [9].

Many countries have amended their policies to minimize – or ban – the use of chemical pesticides, in order to promote biopesticides [1], which can be defined natural products from living organisms including plants that limit or reduce pest populations [3]. Botanic pesticides are efficient for controlling a wide range of crop pests, they are easily biodegradable, have varied modes of action, and have low toxicity for non-target organisms [11]. They are able to disrupt insect mating or kill certain insect larvae. The protectors incorporated in the plants are pesticide substances produced from genetic material [12].

Azadirachtin is the main substance obtained from extracts of Azadirachta indica, belonging to the Meliaceae family [13] and has bio-insecticidal capabilities [14]. Azadirachtin has a variety of physiological effects on many insect pests, such as antifeedancy [15]. Importantly, the efficacy of Azadirachtin varies depending the plant part, especially when isolated from leaves or seeds [16]. Neem plants are growing on various continents [14]. Many investigations on male rats have indicated that oral administration of Azadirachtin was observed to lower sperm viability and serum testosterone levels [17].

On the other hand, plant-based insecticides derived from garlic extract, can be used to repel crop pests. In agriculture, it is well known that the odor of garlic (Allium sativum) can be utilized to repel numerous pests in plants. Garlic includes beneficial chemical substances such as essential oils, allyl disulfide, and allicin, which are good pest repellents. Furthermore, allicin is a chemical that gives garlic its characteristic odor [18].

The present work aims to comparing the toxic effects of the Imidacloprid, Azadirachtin, and an aqueous extract of garlic as bio-insecticide on male fertility and stress oxidative status in male Wistar rats.

MATERIALS AND METHODS

Animals. This study was carried out on 56 male *Wistar* rats *(8 in each group)*, each, about four months old and weighing between **250 and 300 g.** They were purchased from the Pasteur Institute in Algiers, Algeria. Rats were placed in polypropylene cages (230*430 mm) at the animal housed in the biology department at Badji Mokhtar University under natural conditions of photoperiod at a temperature of 23±2°C. Animals were provided with a diet based on Corn, Barley, Soy, Phosphate, Limestone, Cellulose, Minerals and Vitamins from the eastern livestock feed unit, Oued Fragha – Guelma, Algeria) and free access to water *ad libitum*.

Plant extraction. In the manufacturing process of the aqueous garlic extract according to [19]. The method involved weighing and pulverizing peeled garlic bulbs using an electric grinder. The resulting grind was homogenized with 100 mL of distilled water in a high-speed blender for 15 minutes, followed by swirling for 24 hours for maceration. After filtration using a Wattman no.3 paper, the resultant aqueous extract of garlic was stored at 3±1°C for subsequent use.

Treatment. After the adaptation period (20 days), rats were weighed and divided into 7 groups (8 rats in each group), and the rats were daily supplied with treated diet for 6 weeks as follows:

Group 1 (G1): the control group received a standard diet

Group 2 (G2): treated with 1/30 LD50 of Azadirachtin

Group 3 (G3): treated with 1/15 LD50 of Azadirachtin

Group 4 (G4): treated with 1/30 LD50 of Imidacloprid

Group 5 (G5): treated with 1/15 LD50 of Imidacloprid

Group 6 (G6): treated with 1/30 LD50 of the garlic aqueous extraction

Group 7 (G7): treated with 1/15 LD50 of the garlic aqueous extraction

For all experimental treatments, the bio-insecticide Azadirachtin, the aqueous extract of garlic, and the chemical pesticide Imidacloprid were mixed with a standard diet. Imidacloprid has an LD50 of 450 mg/kg [20], Azadirachtin has an LD50 of >5000 mg/kg [21], and garlic has an LD50 of 32g/kg [22].

Collection of blood, sperm and testes. Sample collection. At the end of the 6-week treatment period, rats were sacrificed. A volume of 3 ml of blood was collected dry tubes, which were centrifuged at 3000 rpm for 15 minutes at 4 °C to separate the serum for testosterone analysis. Testes were prevailed and weighted using a KERN PRS 320-3 precision balance (d=0.1mg) and stored at -20°C for evaluation the oxidative stress parameters.

Hormonal assays. The enzyme-linked immunosorbent assay (ELISA) method, as described in the manual for the commercial kit (produced by DGR Instruments GmbH, Germany), was used to measure the serum testosterone concentration.

Sperm Analysis. Sperm parameters including concentration, mobility, curvilinear velocity (VCL), velocity straight-line path (VSL), beat cross frequency (BCF), and lateral head displacement (ALH) were measured using CASA (Computer-Assisted Sperm Analysis) method with Sperm Class Analysis (SCA®, Microptic S.L, Barcelona, Spain. The sperm was diluted in a physiological solution of 0.9% NaCl at 37 °C. 5 µl of the mixture were placed in the Gold-Cyto counting chamber and the examination was carried out under a NIKON® ECLIPSE Ci microscope (Nikon E200-LED) using a x40 objective with negative-phase contrast and phase condenser contrast.

Vitality. The hypo-osmotic swelling test is used to assess the vitality and membrane integrity of spermatozoa, in particular the morphological changes of their tail. A solution containing sodium citrate and fructose is prepared and frozen for 10 minutes. After thawing, the solution is well mixed. To perform the test, 1 ml of the solution is placed in a water bath at 37°C for 3 minutes. Then, 0.1 ml of semen fluid is added and the whole is incubated at 37°C for 30 minutes. After observation under a microscope at a magnification of x40. The percentages of live and dead spermatozoa were manually calculated based on the analysis of 100 counted spermatozoa.

Oxidative stress assays. The preparation of the tissue homogenate was carried out by grinding one gram of tissue with 9 ml of a buffer solution called Tris-buffered saline (TBS) containing 50 mM Tris, 150 mM NaCl and a pH of 7.4. The cell suspension obtained was centrifuged at 3000 rpm for 15 minutes and the supernatant was stored at -20°C. This supernatant was used for the assays of the oxidative stress parameters.

The dosage of reduced glutathione (GSH) was carried out according to the method of Weckbecker & Cory [23]. This assay is based on the measurement of the optical absorbance of 2-nitro-5-mercapturic acid, which is formed by the reduction of 5,5'-dithiobis-2-nitrobenzoic acid (reagent of Ellman, DTNB) by the sulfhydryl groups (-SH) present in GSH.

The malondialdehyde (MDA) assay was performed on testicular samples. For the preparation of the homogenate, 500 mg of tissue were cold ground in the presence of a phosphate buffer solution. MDA is a product formed during the degradation of polyunsaturated fatty acids by free radicals. In our study, MDA levels in hepatic and testicular tissues were assessed according to the method of [24]. The assay is based on the reaction between MDA and thiobarbituric acid (TBA) at high temperature and in acidic medium, forming a colored pigment absorbing at 530 nm, which can be extracted by organic solvents such as butanol.

The enzyme activity of glutathione peroxidase (GPx) was assayed on and testicle samples. For the preparation of the homogenate, 500 mg of each tissue were cold ground in the presence of a TBS solution. The enzymatic activity of GPx was measured according to the method of [25]. This method is based on the reduction of hydrogen peroxide (H2O2) in the presence of reduced glutathione (GSH), which is converted into oxidized glutathione (GSSG) by the action of GPx.

Protein assay. The protein concentration is determined using the method of [26] which uses Coomassie blue (G 250) as a reagent. Coomassie blue reacts with amino groups (-NH2) of proteins to form a blue colored complex. The intensity of the blue color reflects the degree of ionization of the acid medium and corresponds to the concentration of proteins.

Statistical Analysis. All results were expressed as means ± SEM. The statistical analysis was performed by computing *t*-student pairs between the control group and each treated group. The statistical analysis was carried out using GraphPad Prism Version 5.

RESULTS AND DISCUSSION

Synthetic pesticides represent a significant cost for smallholder f armers and may not be widely available [27]. The purpose of this study is to evaluate the toxicity of a chemical insecticide to a plant bio-insecticide isolated from Neem *(Azadirachta indica*) and the aqueous extract of garlic on male fertility in *Wistar* rats after 6 weeks of treatment.

Effects of the 6-week treatment on rats. No death was recorded during the 6-week oral treatment of the rats, and no sign of adverse effects was noticed.

Effects of the treatment on the weight of testes and epididymis. The results of the experiment are presented in Table (1) and revealed a significant reduction in the mass of the testes in the rats which received one of the two tested doses of Imidacloprid, as compared with their control relatives. Conversely, the mass of the testes of the rats supplied with the garlic extracts was increased. There was no significant decrease in the weight of the testis and epididymis in the group treated with Azadiracthin as compared to the untreated rats.

Actually, pesticides have endocrine-affecting qualities that can disrupt the male reproductive system by upsetting the hypothalamic-pituitary-testicular axis [28]**.** As its name suggests, this axis is made up of the hypothalamus, pituitary gland, and testes. Multiple levels of regulation are in place for spermatogenesis, and new sperm function tests use molecular diagnostics [29].

It is known that testicular mass is a good clinical marker of male reproductive function [30]. It is suggested that the decrease in testis weight is probably due to two effects: the anti-spermatogenic is the first, which can decrease the diameter and number of spermatogonia, spermatocytes, and spermatids in the seminiferous tubules of the testis. The second one is the anti-androgenic effect, which reduces the number of sperm in the tubules of the testis [31]. The reduction in testicular mass observed in pesticide-treated rats could be attributed to a decrease in seminiferous tubule size [32] and a halt in spermatogenesis as a result of steroid biosynthesis inhibition; testosterone is a critical component in the normal development and functioning of the testes as it requires continuous androgenic stimulation [6].

Aladakatti et al. found that administering Azadirachtin (1.5 mg/kg body mass) for 24 days resulted in a reduction in the mean testis weight, which corroborated our findings [33]. Similarly, Lonare et al. discovered a substantial reduction in testis weight in rats orally treated with Imidacloprid [9]. It is mentioned that S-allyl cysteine, an active ingredient in garlic, is the most abundant bioactive compound [34]. According to a recent study on his effects, the garlic therapy had no influence on the Fischer rat's

testis weight ratio [35].

The effect of the chemicals on reproductive parameters and testosterone concentration. Sperm concentration, viability, and velocity in the tail of the epididymis showed that sperm concentration was significantly decreased in the rat group treated with the lowest dose of Azadirachtin ($p<0.01$) (Table 2). These reproduction parameters were significantly decreased ($p<0.001$) in rats treated with the highest Azadirachtin dose. A similar finding was noted on the vitality (%) for the rats that were exposed to Imidacloprid in comparison to controls (Table 3). The motility of the sperm was also decreased at the highest dose of Azadirachtin, and with the two doses of the chemical pesticide imidacloprid, as compared to the control. As shown in Table (2), the number of viable spermatozoa decreased significantly in the Imidacloprid-treated animals as compared to the control group. The same result was observed in the curvilinear velocity (VCL) and average path velocity (VAP), which showed a significant reduction ($p<0.001$) in the G4 and G5 groups treated with imidacloprid compared to the control group (Table 3).

The reduction observed in sperm concentration and vitality in the groups treated with the chemical pesticide could be reasonably attributed to oxidative stress induced by the pesticides; it may also be explained by the drastic changes in testicular tissue generated by the depletion of type A turnover spermatogonia caused by Imidacloprid exposure, which plays an important role in the proliferation phase of spermatogenesis [36]. In addition, Imidacloprid induces an increase in ROS production in rats [9]. These free radicals may damage the testicular germ cells, resulting in changes in spermatogenesis that reduce the amount of spermatozoa [37]. Similar to this, it was discovered that oxidative stress causes DNA breakage, membrane lipid peroxidation, and apoptosis, all of which affect spermatozoa function [38]. The decrease in sperm motility measures found in our study appears to be an indication that sperm motility cannot be acquired or maintained. Since the spermatozoa tail is a crucial organ for spermatozoa motility, these results might be explained by disruptions to its morphology [39]. As a result, it was found that sperm flagella morphology significantly correlated with changes in sperm motility [39]. Alternatively, it might be due to any contraceptive drug that directly affects spermatozoa by changing their enzymes [40].

These findings back up those of Mohamed et al., who discovered a substantial sperm abnormality in adult rats subjected to 1 mg/mL/kg body weight of Imidacloprid for 65 days [41]. At 90 mg/kg, Imidacloprid oral 15-day therapy resulted in a reduction in sperm count, motility, and vitality in male rats [8]. In another study conducted by Aladakatti et al., the findings of Azadirachtin therapy at a dosage of 1.5 mg/kg body weight indicated a substantial reduction in sperm count and motility [33].

Osonuga et al. have reported that there were increases in sperm count, motility, and viability in the treatment of Wistar rats with garlic extract for 21 days [42]. Also, when Wistar rats were given garlic extract at a dose of 250 mg/kg for 15 days, there was an increase in sperm count and a decrease in sperm abnormalities [43]. In other research, after 35 days of administration of 400 mg/kg of garlic bulb aqueous extract to rats, it was found to improve sperm motility and viability in the Wistar rat [44].

It can be explained that garlic contains organosulfur compounds that contribute to the formation of reactive sulfur species [45]. The results are most likely due to the nutrients and sperm-producing precursors found in garlic. The active components from different chemical groups may be responsible for improving the quality and quantity of sperm production [46].

TABLE 1
Mean body weight, testicular, and epididymis absolute weights (g) in treated rats compared to the control. Results are expressed as (Mean ± SD, N = 8).

Groups / Mass	Control rats	Rats treated with Azadiracthin		Rats treated with Imidacloprid		Rats treated with Garlic Extracts	
	G1	G2 1/15 LD50	G3 1/30 LD50	G4 1/15 LD50	G5 1/30 LD50	G6 1/15 LD50	G7 1/30 LD50
TM (g)	1.681 ± 0.041	1.662 ± 0.070	1.650 ± 0.029	1.556 ± 0.148	1.549 ± 0.060*	1.717 ± 0.073	1.730 ± 0.041
EM (g)	0.631 ± 0.044	0.600 ± 0,044	0.561 ± 0.044	0.542 ± 0.139	0.520 ± 0.060	0.631 ± 0.054	0.640 ± 0.021
IBM (g)	260.3 ± 4.979	261,6 ± 5.012	261.1 ± 6.266	261.1 ± 5.436	260.4 ± 2.387	260.4 ± 6.093	262.6 ± 6.948
FBM (g)	294.5 ± 6.782	296,4 ± 5.208	294.8 ± 6.296	296.9 ± 6.232	298.3 ± 5.392	291.9 ± 7.200	286.6 ± 5.423
MG (g)	34.2	34.8	33.7	35.8	37.9	31.5	24

NS: at $p>0.05$; * Significantly different from control at $p<0.05$ level; ** Significantly different from control at $p<0.01$ level; *** Significantly different from control at $p<0.001$ level; TW: Testes mass; EM: Epididymis mass; IBM: Initial body mass; FBM: Final body mas; MG: Mass gain.

Similar results in velocity parameters were obtained when rats were given Imidacloprid at dosages of 112 and 225 mg/kg orally for 60 days, resulting in a significant increase in aberrant sperm velocity [10]. The reduction in velocity parameters (VSL, VAP, and VCL) at pH 6.2 and 5.2 [47] may better show the influence of pesticides on environmental pH. In other hand the results can be explained by the role of the mitochondria as an energy generator, which is reflected in the sperm motility, velocity characteristics (VCL, VSL, and VAP), ALH, and BCF. It is possible that mitochondrial dysfunction, which lowers the energy needed for flagella movement [39], is the cause of the negative effects on the sperm motility parameters found in our results. Additionally, sperm motility, velocity, lateral head displacement, and beat cross frequency all depend on Na,K-ATPase α4 isoform activity [48]. The Na+-K+ ATPase is a universal transmembrane protein, and only sperm and germ cells express its α4 isoform (ATP1A4) [49]. Additionally, ATP1A4 controls signaling molecules in the raft and non-raft portions of the sperm plasma membrane to control activation, sperm-oocyte contacts, hyperactivation, and signaling molecules involved with capacitation. Abnormalities in these sperm characteristics have long been utilized as a predictor of male fertility [50]. Pesticides may block or deactivate the Na,K-ATPase or the α4 isoform, which explains why.

The level of testosterone was significantly reduced in the rats which were supplied with food contaminated by Imidacloprid or Azadirachtin groups ($p<0.001$) as compared with control rats. This drop was more severe for rats exposed to Imidacloprid in comparison to untreated animals. The level of this hormone was increased in the rats treated with the highest dose of garlic extract in comparison to the control rats ($p< 0.01$).

The sperm tail destruction and the drop in testosterone levels, indicating testicular dysfunction, was observed in mice treated with pesticides [51]. Furthermore, the reduced density of epididymal sperm may be linked to a change in androgen metabolism [52].

Pesticides, due to their affinity for androgen receptors, can stimulate or block these receptors, inhibiting their function [7]. They are likely to have an effect on the hypothalamus-pituitary axis, which produces the pulsatile gonadotropin-releasing hormone (GnRH), which is responsible for gonadotropins luteinizing (LH) secretion [53]. LH stimulates Leydig cells to synthesize testosterone; consequently, a reduction in LH may mean a reduction in testosterone levels [54]. Furthermore, endocrine-disrupting pesticides can interfere with normal hormone production, secretion, transport, metabolism, binding, or removal [55]. Many studies have found that pesticides impede non-specific esterase activity in Leydig cells, resulting in lower testosterone synthesis [10]. According to Moreira et al., the lowering of testosterone levels is a common impact of all kinds of pesticides on Leydig cells, and two main mechanisms appear to be responsible for this action [32]. The chemicals' first endocrine disruptive effects produce the inhibition of genes involved in the steroidogenic process, and their presence in organisms causes elevated levels of ROS, which in turn cause mitochondrial damage [32].

These findings are consistent with those of Mohamed et al., who discovered that blood testosterone levels were considerably lower in adult rats treated with Imidacloprid at a dosage of 1 mg/mL/kg body weight for 65 days [41]. Imidacloprid exposure can impair the proper functioning of animal endocrine systems by interfering with endogenous hormones [7]. Auta & Hassan discovered that giving male albino mice (5, 50 and 100 mg/kg body weight) dosages of commercial Azadirachtin insecticide extract for 6 days resulted in no significant variations in testosterone levels across experimental groups [56].

Similar outcomes have been noted in male Bouscat rabbits receiving daily doses of 3, 9, and 27 mg/kg of garlic extract for 8 weeks [57]. Similar outcomes were shown when Sprague-Dawley rats were given garlic oil at 80 mg/kg/day for five days a week for 90 days [58].

TABLE 2
Variation of the mean reproduction parameters in the control Azadirachtin treated groups ($\bar{X}$±SD, n=8).

Groups	G1: Control	Azadirachtin	
		G2: 1/30 LD50	G3: 1/15 LD50
Concentration (10^6/ ml)	171.74 ± 4.620	164.222 ± 1.56 *	153.582 ±2.72 ***
Motility (%)	87.29 ± 2.698	87.14 ± 2.315	76.27 ±2.821 ***
Vitality (%)	82.80 ± 3.033	81.00 ± 2.550	73.00 ± 3.082
VCL (µm/s)	88.40 ± 5.520	83.41 ± 3.27	81.78 ± 2.38
VSL (µm/s)	16.29 ± 1.248	17.89 ± 1.410	17.19 ± 1.421
VAP (µm/s)	40.23 ± 0.460	40.83 ± 1.134	40.49 ± 0.822
ALH (µm)	4.764 ± 0.229	4.520 ± 0.185	4.408 ± 0.157
BCF (Hz)	4.542 ± 0.102	4.544 ± 0.083	4.284 ± 0.070**

TABLE 3
Variation of the mean reproduction parameters in the control and Imidacloprid treated groups ($\bar{X}$±SD, n=8).

Groups	G1: Control	Imidacloprid	
		G4: 1/30 LD50	G5: 1/15 LD50
Concentration (10^6/ ml)	171.74 ± 4.620	145.012 ±3.45 ***	141.216 ±4.60 ***
Motility (%)	87.29 ± 2.698	58.57 ±0.691 ***	43.79 ±2.776 ***
Vitality (%)	82.80 ± 3.033	57.00 ± 3.391 ***	53.60 ± 4.848 ***
VCL (µm/s)	88.40 ± 5.520	76.82 ± 3.32 *	75.17 ± 2.78
VSL (µm/s)	16.29 ± 1.248	16.29 ± 1.849	17.43 ± 1.156
VAP (µm/s)	40.23 ± 0.460	37.07 ± 0.668 **	36.04 ± 1.542 ***
ALH (µm)	4.764 ± 0.229	4.138 ± 0.156	3.906 ± 0.478 **
BCF (Hz)	4.542 ± 0.102	4.536 ± 0.0763 ***	4.220 ± 0.129 ***

TABLE 4
Variation of the mean reproduction parameters in the control and groups with Garlic aqueous extract ($\bar{X}$±SD, n=8).

Groups	G1: Control	Garlic Aqueous Extract	
		G6: 1/30 LD50	G7: 1/15 LD50
Concentration (10^6/ ml)	171.74 ± 4.620	172.68 ±3.07	176.122 ±4.22
Motility (%)	87.29 ± 2.698	86.41 ±2.057	87.46 ±4.658
Vitality (%)	82.80 ± 3.033	83.00 ± 2.00	87.60 ± 1.949
VCL (µm/s)	88.40 ± 5.520	86.19 ± 2.18	88.52 ± 3.53
VSL (µm/s)	16.29 ± 1.248	15.08 ± 0.797	12.56 ± 1.403 ***
VAP (µm/s)	40.23 ± 0.460	36.51 ± 2.076 **	41.05 ± 1.951
ALH (µm)	4.764 ± 0.229	4.306 ± 0.219	4.408 ± 0.337
BCF (Hz)	4.542 ± 0.102	4.016 ± 0.089	4.028 ± 0.221

NS: Not significantly different from control at p>0.05
* Significantly different from control at p<0.05 level
** Significantly different from control at p<0.01 level
*** Significantly different from control at p<0.001 level

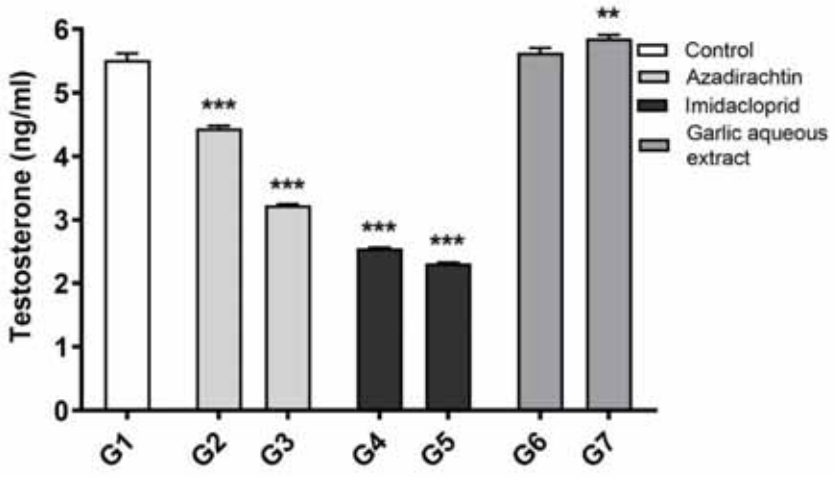

FIGURE 1
Mean testosterone concentration (ng/ml) in treated rats compared to the control group. (Mean ± SD, N = 8).
NS: Not significantly different from control at p>0.05, * Significantly different from control at p<0.05 level, ** Significantly different from control at p<0.01 level, *** Significantly different from control at p<0.001 level.

Effects of the chemicals on the oxidative stress markers. The MDA levels were significantly higher in rats supplied with Imidacloprid-contaminated food and G3 treated with the highest dose of Azadirachtin than in the control group (p<0.001). They were considerably higher and different from the control group in the Azadirachtin treatment group on the lower dose (Figure 1). When compared to the control group, they were significantly lower in

the garlic extract group at the highest dose, particularly in G7 treated with the highest dose.

In comparison to the control group, GSH and GPx levels decreased significantly ($p<0.001$) in both Imidacloprid groups. GSH did not show any changes in other groups (Figure 2). GPx levels were slightly decreased in G2 and showed a significant drop ($p>0.05$) in G3, which was treated with Azadirachtin at the highest dose. But there were not significantly higher levels in the garlic extract groups compared to the control group (Figure 2).

Malondialdehyde (MDA) is an essential indicator of oxidative lipid damage since it is a key oxidative product of peroxidized polyunsaturated fatty acids in tissue [59]. The MDA levels in the Azadirachtin and Imidacloprid treatments were somewhat significantly higher, while they were lower in the G-Ext groups.

Our findings are congruent with those of Bal et al., who observed a rise in MDA levels in the testes following 90 days of oral Imidacloprid administration at a dosage of 8 mg/kg to male rats [6]. Regarding the garlic effects, rats were given garlic bulb juice for 4 weeks at doses of 60 and 120 mg/kg, a decrease in the activity of semen MDA [60]. Similar outcomes were obtained when Wistar rats were given 250 mg/kg of garlic extract for 15 days [43]. According to Lotfi et al., the allicin in garlic can suppress LPO, trap free radicals, and lower blood lipid levels [61]. Other research has demonstrated that the use of garlic can reduce oxidative stress [62].

Because testicular tissue divides rapidly and is extremely vulnerable to free radicals and oxidative stress [61]. Glutathione peroxidase activity levels increased in the groups treated with the aqueous extract of A. indica leaves, according to Christian et al.'s findings. Plant extracts with significant phenol and flavonoid content have demonstrated antioxidation properties [63]. Our findings may have been influenced by the presence of these phytochemicals. It is now that Azadirachtin can increase cellular survival under severe inflammatory and oxidative stress circumstances by shielding LPS-induced cells from inflammatory-related metabolic and molecular alterations and mitochondrial malfunction [64].

Imidacloprid induces an increase in ROS production [65]. The creation of excessive ROS generation may modify the cellular antioxidant defense system and, as a result, impair susceptibility to oxidative stress, which may be accountable for Imidacloprid's harmful impact [9]. ROS formation is believed to cause DNA damage, protein degradation, and lipid peroxidation (LPO), all of which cause harm to numerous essential tissues [9]. These extremely reactive free radicals deplete antioxidant defense mechanisms, which can lead to cell instability and oxidative damage to the cell membrane [6].

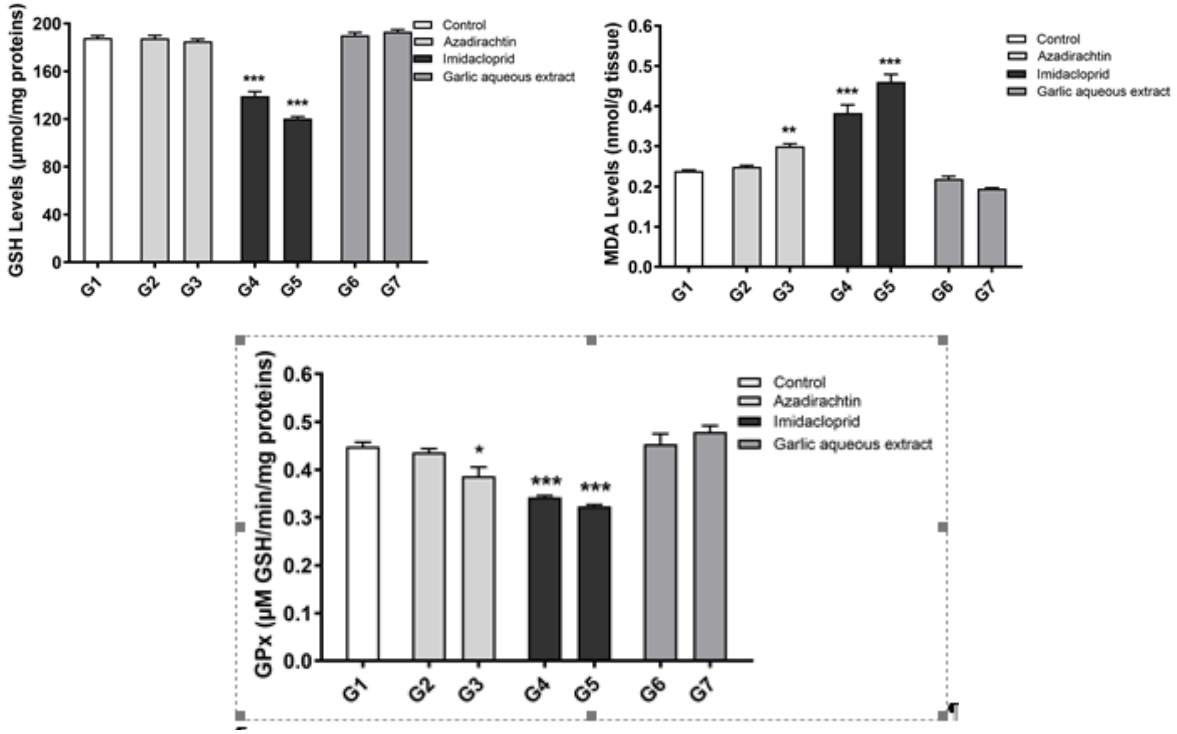

FIGURE 2

The mean Testicular levels of malondialdehyde (MDA) (µmol/g tissue); glutathione (GSH) (µmol/mg proteins) and glutathione peroxidase (GPx) (µM GSH/min/mg proteins) of treated rats compared to control. (Mean± SD, N = 8).

NS: Not significantly different from control at $p>0.05$, * Significantly different from control at $p<0.05$ level, ** Significantly different from control at $p<0.01$ level, *** Significantly different from control at $p<0.001$ level.

In previous research, male adult rats treated with Imidacloprid at a dosage of 8 mg/kg for 90 days had a significant drop in GSH levels [6]. There were also some significant findings when GSH and GPx levels in the testis were reduced by Imidacloprid at 90 mg/kg

Garlic also reduced oxidative stress, neutralized free radicals, improved spermatogenesis, and increased fertility [66]. Due to its antioxidant characteristics, garlic is a wonderful choice for this [61]. Numerous studies show that garlic has antioxidant properties; an increase in GSH and GPx activities was seen in Wistar rats given garlic extract at a dosage of 250 mg/kg once daily for 15 days in order to study the damage Adriamycin caused to the testicles [43]. There was also an increase in antioxidant protection in the testes of rats treated with aqueous garlic extract at 400 mg/kg for 35 days [44]

CONCLUSION

Exposure of rats to two doses of Imidacloprid and Azadirachtin by alimentation for 60 consecutive days may induce reproductive toxicity in male rats, which is manifested in reduced organ weight, decreased sperm concentration, motility, and velocity, and perturbation in the stress oxidative parameters. The treatment with the aqueous extract of garlic is less toxic in our experimental conditions.

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Ethical approval. Experimental procedures were performed according to the natural guide for the care and use of laboratory animals and approved by the animal sciences committee at Badji Mokhtar University in Algeria.

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ASSESSMENT OF SUB-ACUTE ORAL ADMINISTRATION OF AN AQUEOUS EXTRACT OF *Drimia Maritima's* BULB AND LEAVES ON LIVER AND KIDNEY FUNCTION IN ALBINO WISTAR RATS

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ABSTRACT

Based on the numerous medicinal benefits of the plant *Drimia maritima* (L.) Baker (*Asparagaceae*) and its use in the environment, this study examined the risk of this use on non-target species by examining the phytochemical and toxicological profile of the aqueous extract of *D. maritima* bulb and leaves on the liver and kidney function of Wistar rats. Seven groups of rats (n = 6) were utilized for subacute studies, including a control, three doses of an aqueous extract of *D. maritima* bulb (DBAE), and three doses of an aqueous extract of *D. maritima* leaves (DLAE), each at a dose of 98, 49, and 24.5 mg/ kg. The findings showed that both plant extracts include a large amount of phenolic chemicals, which provide them great antioxidant capability. Additionally, only the high oral dosage of DBAE showed a modest rise in hepatic and renal enzymes, even though the antioxidant enzyme levels (GST, GPx, and GSH) in the liver and kidney remained steady and hematological parameters did not change. However, none of the measures under study significantly changed because of the three DLAE dosages involved. The antioxidant status of both *D. maritima* extracts is raised. After subacute administration at levels below 98 mg/ kg body weight, DBAE was mostly non-toxic. But at the studied levels, DLAE was safe.

KEYWORDS:
Drimia maritima, Aqueous extract, Phytochemistry, Hematological parameters, Oxidative stress, Biochemical parameters

INTRODUCTION

Drimia maritima (L.) Baker, commonly known as *(Squill),* belongs to the family *Asparagaceae*, it is a species that grows spontaneously along North Africa's coastline and in abundance in the Seraidi area in the Algeria east. It is distinguished by its ovoid bulb of 15 to 30 cm long and its long lanceolate leaves pointed at their tips [1], is a species polymorphic, with several forms and variants. Many plants, including *D. maritima*, are renowned for their medicinal potential and interesting therapeutic powers [2]. In Algeria, the red variety has been the subject of a severe collection to hunt for novel bioactive chemicals; it is a key player much like many other conventionally used therapeutic herbs. Traditional therapists admitted *Squill* as a medication for the treatment of a variety of illnesses, including as respiratory conditions, digestive issues, cardiac disorders, joint and skin issues, and cancer, this was stated by [3]. It is also utilized as an aqueous extract to cleanse the blood, through the usage of its numerous derived biocompounds [4]. Although it has traditional therapeutic uses, *D. maritima* also has toxicological properties. Indeed, the liver and kidneys are essential organs that contribute to the detoxification of organisms, as they are the target organs for all xenobiotics. This is done by ensuring the metabolism and elimination of xenobiotics and their metabolites [5]. Thus, several authors have suggested that high doses of *D. maritima* or prolonged consumption cause gastrointestinal and urinary disorders due to certain cardiac glycosides derived from this species, such as scilliroside, which is present in almost every part of the plant, including the leaves and bulb center [6], [7].

It is obvious that plants include biologically active substances, and they are also a significant source of flavonoids, tannins, polyphenols, and alkaloids, all of which have potent antioxidant effects and low permanence in the products they are intended to be used in [8]. Chemical analyses in several studies, including [9] and [10], have shown the presence of bufadienolide cardiotonic heteroglycosides (scillarenes, proscillaridins) as the main constituents of

D. maritime, as well as flavonoids, polyphenols, tannins and polysaccharides, thanks to which it possesses its antioxidant [11] and diuretic properties [12] anti-hemolytic [13] cytotoxic [14], [15] antifungal[16] and insecticidal [17].

The use of plant extracts has certain advantages. Biodegradable products from plants are a good alternative that allows producers to protect their crops at a relatively low cost and target specific. The identification of new biodegradable molecules has been developed to reduce the permanence of synthetic compounds and their residues in the environment [18], [19] can be used in both conventional and organic farming, some allow plants to withstand abiotic stresses and are generally less toxic than their chemical counterparts. Although they often have a reputation for being less effective than their chemical counterparts, biopesticides are the subject of increasing interest from farmers, particularly in the context of integrated pest management strategies.

Currently, study on safety profile of the plant extract is lacking. Therefore, in the present study, we evaluated the subacute toxicological effects of the aqueous extract of the bulb and leaves of this plant on the liver and kidney of Wistar rats by the evaluation of biochemical and alterations and oxidative stress in these tissues.

MATERIALS AND METHODS

Plant material and their collection. *Drimia maritima* (L.) (*Asparagaceae*) is a bulbous plant with rosette-shaped, thick, and soft leaves that appear in early winter. The bulb is wide, red, and weighs over than 3 kg [1]. In the current experiment the fresh whole plant (bulbs and leaves) of *D. maritima* were harvested from Seraidi Province in the north of Annaba, Algeria (at 36° 55′ 21.9″ North and 7° 43′ 30.4″ East) between November and December. The species was botanically identified by Dr Hamel Tarek and Dr Slimani Rachid, taxonomists in the Department of Botany, University of Annaba.

Plant extraction. The leaves and bulb of the collected plant were cleaned with distilled water, sliced into pieces, dried, and turned into powder. The *D. maritima* bulb extraction (DBAE) was made by combining 100 g of powdered bulbs with 1000 ml of distilled water (ratio 1/10 w/v) and bringing it to a boil (100 °C) in a beaker for 15 to 20 min. For the *D. maritima* leaf extract (DLAE), 100 g of finely ground *D. maritima* leaves are added to a liter of boiling distilled water at a ratio of 1/10 (w/v) and let's infuse for 30 min.

After cooling, the two extracts are filtered through Whatman filter paper and dried in an oven at 40 °C, then each extract's yield was determined before keeping them in 4 °C until use [20].

Phytochemical analysis and antioxidant potential of aqueous extract of (DBAE) and (DLAE). The quantification of total phenolic content in both aqueous extracts was performed using the Folin-Ciocalteu reagent [21]. A 0.2 ml of both diluted aqueous extract and 1 mL diluted Folin's reagent (1:1:10), is incubated for 4 min before an 0.8 ml of sodium carbonate solution (7.5%) is added. The absorbance is measured at 765 nm after 2 hours of incubation. The calibration line was performed with gallic acid. The data are given as milligram equivalent of gallic acid per gram of extract (mg EAG/ g extract).

The quantification of flavonoids in both aqueous extracts was performed by the aluminum trichloride ($AlCl_3$) colorimetric method [22]. The method consists of using the $AlCl_3$ methanolic solution (2%). The absorbances were measured at 430 nm. Values are given as milligrams equivalent of quercetin per gram of extract (mg EQ/ g extract).

Using the DPPH assay, we evaluated the two *D. maritima* extracts' antioxidant capacity [23]. This testing repeated three times makes use of a redox reaction with the radical 2,2-Diphenyl-1-picrylhydrazyl. After 30 min of incubation at room temperature, the absorbance was measured at 517 nm and the activity of scavenging free radicals was calculated using the equation below:

radical scavenging activity (%) = (Abs control – Abs sample/ Abs control) x 100

Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were employed as positive controls, on the identical experimental design.

Animals. Healthy male rats of the Wistar albino strain weighing (210 – 230 g) were used for the experiments. They were purchased from Pasteur Institute of Algiers. Before the launch of the experimental study, the rats were acclimated for two weeks in clean polypropylene cages under a well-aerated room at a temperature of 26 ± 2 °C, and relative humidity of 50 ± 5%, and subjected to natural photoperiod (12 h/ 12 h). The animals had free access to water and standard pellets.

Experimental procedures. After acclimating for two weeks, the experimental animals were divided into seven groups of six rats each (n = 6).

Group 1 (control group) received 1 mL of distilled water via gastric intubation one time daily for 4 weeks.

Groups 2, 3 and 4 administered with the aqueous extract of D. maritima bulb at three doses (98, 49 and 24.5 mg/ kg bw, respectively for 4 weeks) via oral gavage.

Groups 5, 6 and 7 administered with the aqueous extract of D. maritima leaves at three doses (98, 49 and 24.5 mg/ kg bw, respectively for 4 weeks) via oral gavage.

The dosages utilized were selected in accordance with the results of a prior research [1], which

demonstrated that the LD_{50} of *D. maritima* powder is a dose of 490 mg/kg bw.

During the period of the sub-acute toxicity study the animals were observed for any indications of toxicity, and the body weights of each animal were measured daily on an electronic weighing balance, and then calculated for all groups. All animals were scarified 4 weeks after the start of the trial by cervical decapitation. Blood was collected from each animal for hematological analysis in EDTA tubes, and in polyethylene heparin tubes for biochemical analysis. Liver and kidneys were also removed, rinsed in saline solution, and weighed before being kept at -20 °C until use.

Assessment of hematological and biochemical parameters. After the collecting of blood in tubes, blood samples were analyzed for determination of hemoglobin (HB) concentration, red blood cell count (RBC), white blood cell count (WBC), platelet (PLT) using (BioSystems S.A. Costa Brava, Spain). Biochemical analyses were performed using a centrifuge at 3000rpm for 10 min at 4 °C. The collected serum was then analyzed with (BioSystems S.A. Costa Brava, Spain) for measuring levels of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), Lactate dehydrogenase (LDH), total bilirubin (TB), albumin (ALB), urea, creatinine (CR) and uric acid (UA).

Antioxidant enzyme activity assays. 1 g portion from the liver and kidney were taken, and they were homogenized in 3 mL of phosphate buffer (1:3 w/v PBS, pH 7.4) before being centrifuged at 9000 rpm for 15 min at 4 °C. The collected supernatant was frozen (-20 °C) to test oxidative stress and antioxidant indicators (MDA, GSH, GPx, GST).

The protein content of tissues liver and kidney was assessed spectrophotometrically at 595 nm according to [24]. The concentration of proteins was calculated using bovine serum albumin as standard (BSA).

Determination of lipid peroxidation (MDA). The amount of Malondialdehyde (MDA), a by-product of lipid peroxidation, was calculated by measuring the color of the reaction between it and thiobarbituric acid reagent (TBA). Absorbance of the complex was measured using a spectrophotometer at 530 nm [25].

Determination of reduced glutathione (GSH). According to the procedure of [26] modified by [27], the levels of glutathione (GSH) in the liver and kidneys were evaluated. It is a colorimetric method of detecting 2-nitro-5-mercaptobenzoic acid, which has a yellow color and absorbs at 412 nm. This chemical is produced when the thiol groups (SH) reduce the acid 5,5'-dithiobis-2nitrobenzoic DTNB.

Glutathion S-transférase (GST). The activity was quantified following the methods of [28], which require the presence of a combination of CDNB (0.02 M) as a substrate and GSH (0.1 M). The optical density was measured at 340 nm for one min during five min.

Determination of Glutathione peroxidase activity GSH-Px. The activity was determined based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH). The absorbance was read at 412 nm [29].

Statistical analysis. All data analyses were calculated and presented as mean ± standard error of the mean (SEM) by applying one-way analysis of variance (ANOVA) followed by the Tukey's method for multiple comparisons, and the significance level of the results was set at ($p < 0.05$). GraphPad Prism 9.0 software was used to perform the data study.

RESULTS

Evaluation of phytochemistry and antioxidant activity. According to phytochemical investigations of the two aqueous extracts of *D. maritima's* bulb and leaves (Table 1), DBAE contains more polyphenols than DLAE does, with (150.10 ± 2.66 mg EAG/ g DE) and (85.53 ± 2.38 mg EAG/ g DE), respectively. The flavonoid concentration for the leaves and bulbs, respectively, was 11.79 ± 2.46 and 9.80 ± 1.80 mg EG/g DE (Table 1).

The DPPH test's findings are depicted in Figure 1's data. According to these findings, the bulb, and leaves of *D. maritima* have an aqueous extract that has 38.67% and 37.88% scavenging action on the DPPH radical, respectively. When compared to BHT and ascorbic acid (75.98 ± 1.43 and 37.58 ± 2,99 µg/ml, respectively), DLAE and DBAE extract exhibit more potent antioxidant activity, with an IC_{50} value of 96.71 ± 5.60 and 91.93 ± 1.55 µg/ml, respectively.

Values are given as mean ± SEM, (n= 3). (DBAE) *D. maritima* bulb aqueous extracts; (DLAE) *D. maritima* leaves aqueous extract; (DE) dry extract; (EAG) equivalent of gallic acid; (EQ) equivalent of quercetin.

TABLE 1
***D. maritima* extracts yield, total phenolic and total flavonoid content.**

Extract	Yield [%]	Total phenolic [mg EAG/ g DE]	Total flavonoid [mg EQ/ g DE]
DBAE	62.2	150.10 ± 2.66	9.80 ± 1.80

DLAE	34.6	85.53 ± 2.38	11.79 ± 2.46

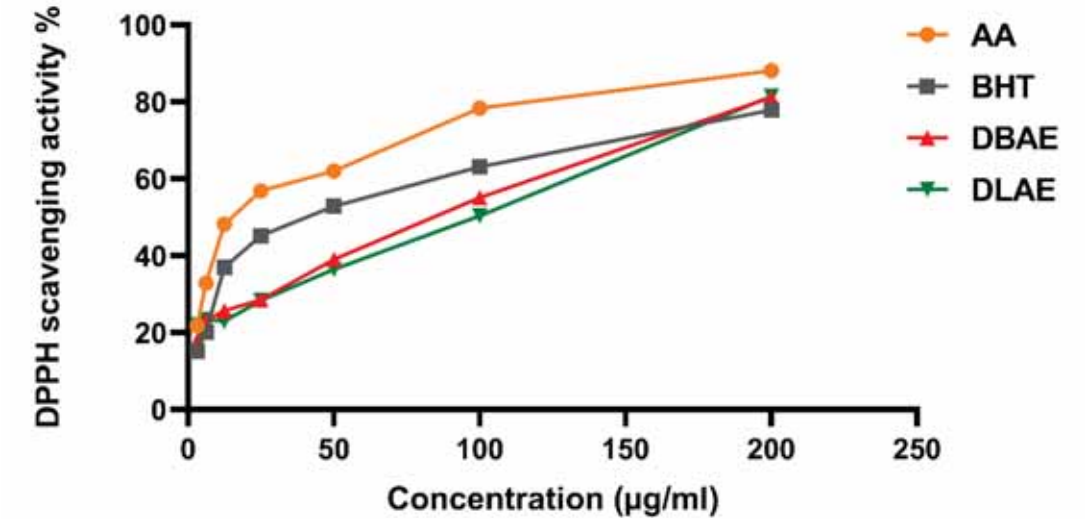


FIGURE 1
DPPH radical scavenging activity of *D. maritima* extracts, ascorbic acid (AA) and butyl hydroxytoluene (BHT) were utilized as positive controls. Each value is represented as the mean of three determinations ± SEM.

Initial and final body weight and relative organ weight of treated and untreated rats. Data on body weight as well as the relative kidneys and liver weights are shown in Table 2. The findings indicate a significantly significant rise in body weight ($p < 0.001$) in the groups given 98 mg/kg of both DBAE and DLAE extracts. However, the groups receiving 24,5 mg/kg of the aqueous bulb and leaf extracts, experienced a negligible increase in body weight growth (+ 55.83 g and + 50.17 g, respectively). Following treatment with DBAE at a high dose, compared to the controls, the relative weight of the kidney decreased significantly ($p < 0.05$). However, there was no discernible difference between control rats and rats that had received the various doses of both extracts in terms of their relative liver weight. Nevertheless, no group of animals displayed clinical intoxication symptoms during treatment.

Effect of *D. maritima* aqueous extract on basal hematological parameters. The result of the impact of DBAE and DLAE on the hematological profile is shown in Table 3. Only the groups that received two extracts DBAE and DLAE at high doses showed a statistically significant increase (10.33 ± 0.46 and 10.63 ± 0.32 $*10^9$/L, respectively) in total WBC. In contrast, RBC, HB, and PLT counts in the control and treatment groups were all within the normal range.

Effect of *D. maritima* aqueous extract on hepatic markers. Rats exposed to 98 mg/kg of DBAE extract, showed substantially greater levels of the liver enzymes AST and ALT than the control group (Figure 2a, b). In comparison to the control, high dosage DBAE caused a highly significant rise in plasma ALP (339.13 ± 25.01 Ul/L) (Figure 2c), which was followed by an important ($p < 0.05$) decrease in serum albumin concentrations (27.87 ± 1.00 g/l) (Figure 2d). Remarkably, the plasma total bilirubin and the LDH enzyme levels between the control and treatment groups did not change significantly (Figure 2e, f).

TABLE 2
Body weight gain and relative kidney and liver weights in control and experimental rats for 4-week.

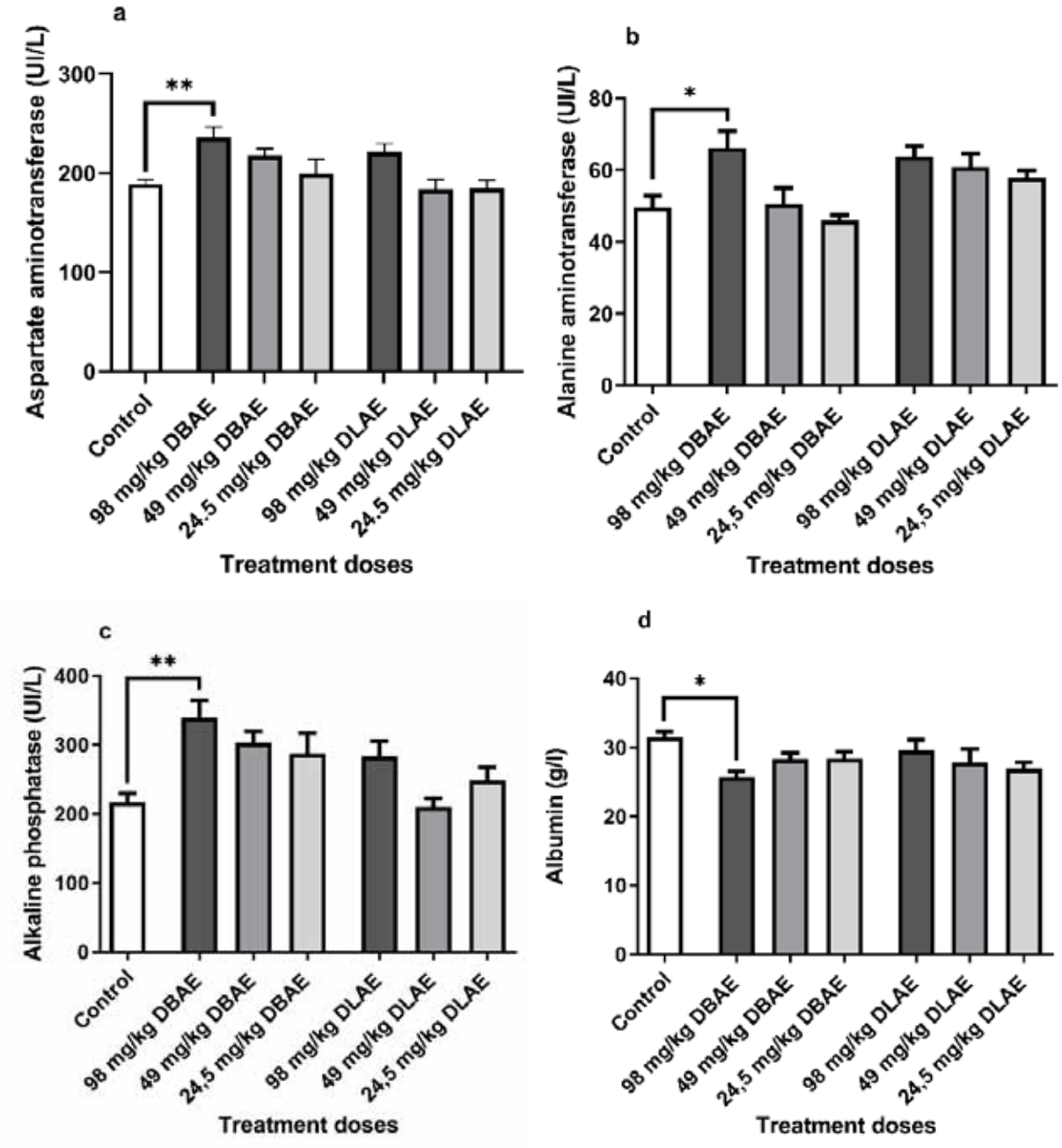
Treatment doses	Initial body weight [g]	Final body weight [g]	Weight gain [g]	Relative kidney weight [g/100 g bw]	Relative liver weight [g/100 g bw]
Control	223.83± 1.97	253.33± 5.49	29.50± 5.78	0.54± 0.02	2.47± 0.12
98 mg/kg bw DBAE	221.50± 0.76	282.16± 5.81***	60.67± 5.42**	0.44± 0.01*	2.38± 0.14
49 mg/kg bw DBAE	222.66± 0.61	273.50± 7.84**	50.83± 7.40	0.51± 0.01	2.24± 0.12
24.5mg/kg bw DBAE	220.33± 0.76	276.16± 6.37***	55.83± 5.91*	0.49± 0.02	2.41± 0.14
98 mg/kg bw DLAE	220.00± 1.06	276.66± 5.37***	56.67± 5.67*	0.46± 0.03	2.54± 0.15
49 mg/kg bw DLAE	223.00± 1.46	261.16± 2.49	38.17± 2.28	0.48± 0.01	1.97± 0.18
24.5 mg/kg bw DLAE	220.66± 0.95	270.83± 3.79**	50.17± 3.26	0.46± 0.02	2.26± 0.06

Values are given as mean ± SEM, for groups of six animals each. *D. maritima* bulb aqueous extract (DBAE); *D. maritima* leaves aqueous extract (DLAE). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control group.

TABLE 3
Hematological variations in control and experimental rats over 4 weeks.

Treatment doses	WBC [*10^9/L]	RBC [*10^{12}/L]	HB [g/dL]	PLT [*10^9/L]
Control	8.37± 0.41	8.60± 0.28	17.88± 0.43	1145.00± 57.53
98 mg/kg bw DBAE	10.33± 0.46*	8.93± 0.21	17.68± 0.19	1388.33± 61.86
49 mg/kg bw DBAE	9.66 ± 0.42	8.72± 0.21	17.10± 0.38	1120.83± 78.30
24.5mg/kg bw DBAE	9.10± 0.37	8.64± 0.13	17.24± 0.25	1093.83± 54.03
98 mg/kg bw DLAE	10.63± 0.32*	8.71± 0.18	17.76± 0.39	1072.16± 61.54
49 mg/kg bw DLAE	9.94± 0.75	9.16± 0.24	17.84± 0.43	1116.33± 50.01
24.5 mg/kg bw DLAE	8.88± 0.36	8.78± 0.17	17.82± 0.43	1026.83± 41.29

Values are given as mean ± SEM, for groups of six animals each. *D. maritima* bulb aqueous extracts (DBAE); *D. maritima* leaves aqueous extract (DLAE); white blood cell (WBC); red blood cell (RBC); hemoglobin level (HB); platelet (PLT). (* $p < 0.05$ versus control group).



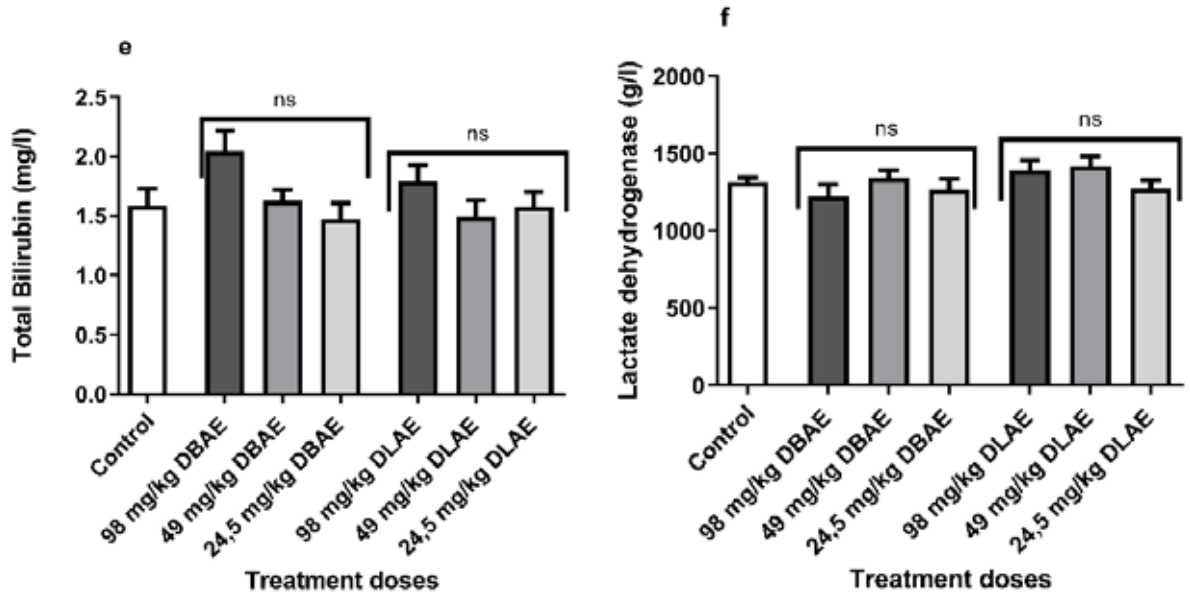
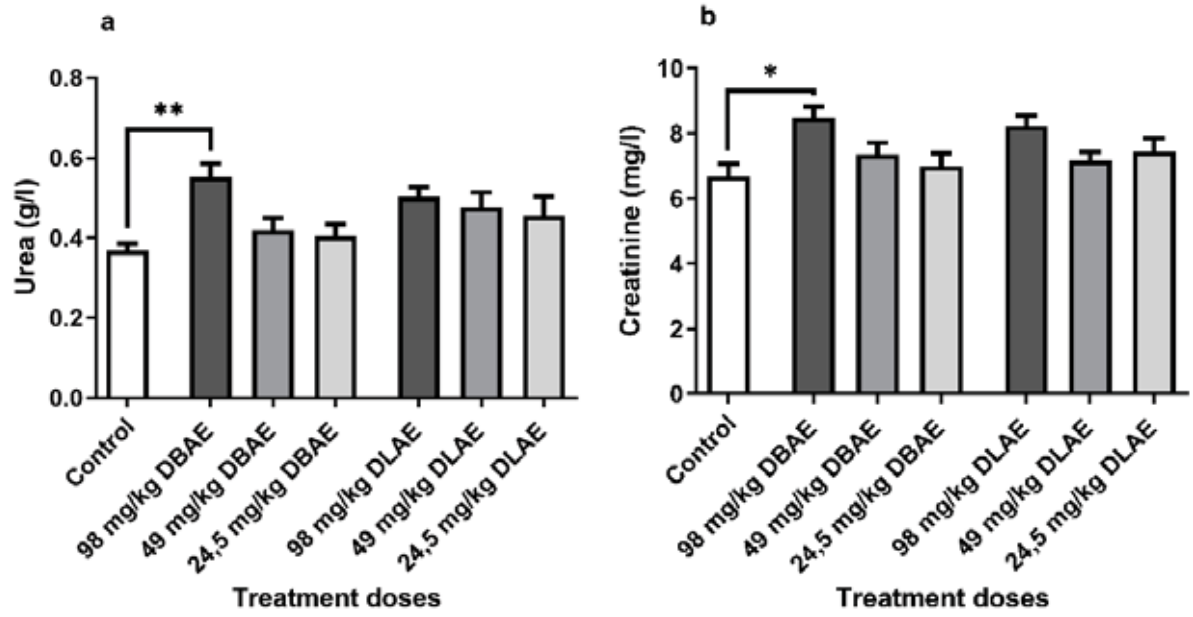


FIGURE 2

Hepatic enzyme levels following treatment of both extracts to animals of different groups over a 4-week period. Values are expressed as mean ± SEM (n = 6). [ns] no significant difference, * $p < 0.05$, ** $p < 0.01$ versus control group.

Effect of *D. maritima* aqueous extract on biochemical markers of renal function. After 28 days of treatment, the serum urea content of rats given high doses 98 mg/kg of DBAE experienced a considerable increase with (0.55 ± 0.03 g/l), while the other groups had insignificantly low levels of urea compared to the control group (0.36 ± 0.01 g/l) (Figure 3a). However, there is a difference seen in the levels of CR and plasma UA, which only significantly rise in the groups that received 98 mg/kg of the plant's bulb extract (Figure 3b, c).

Effect of *D. maritima* aqueous extract on hepatic and renal oxidative stress parameters. Measuring MDA (a marker of the existence of lipid peroxides) in the liver of rats, the values obtained (Table 4) show that administration of DBAE and DLAE at 98, 49 and 24.5 mg/kg for 4 consecutive weeks to the animals presented normal levels of MDA and the content of GSH (Table 4) close to the control in liver tissues.



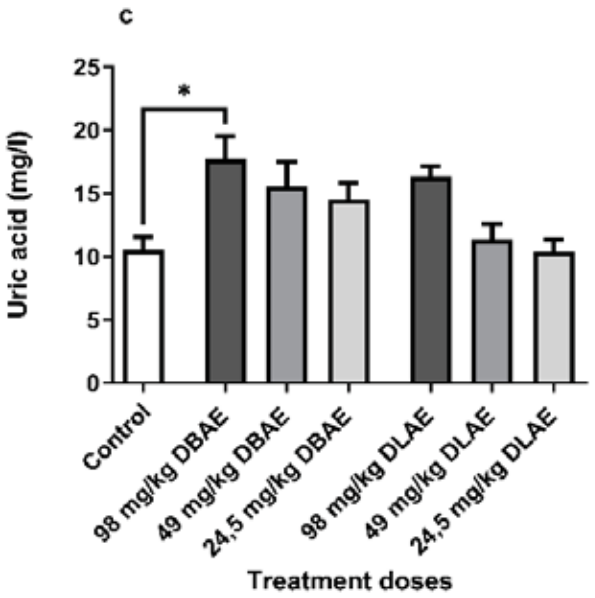


FIGURE 3

Variations in specific serum biochemical kidney function parameters after treatment of both extracts to animals of different groups for a 4-week period. Values are expressed as mean ± SEM. (n = 6). * $p < 0.05$, ** $p < 0.01$ versus control group.

TABLE 4
Response of oxidative stress biomarkers in liver and kidneys tissues of animals from different groups over a 4-week period.

Treatment doses	Liver		Kidneys	
	MDA [nmol/mg prot]	GSH [nmol/mg prot]	MDA [nmol/mg prot]	GSH [nmol/mg prot]
Control	9.47± 1.33	3.30± 0.35	14.78± 1.68	0.71± 0.05
98 mg/kg bw DBAE	8.85± 2.18	3.33± 0.29	13.72± 2.58	0.62± 0.07
49 mg/kg bw DBAE	8.81± 1.69	3.02± 0.27	12.52± 1.79	0.67± 0.07
24.5mg/kg bw DBAE	9.63± 1.78	3.72± 0.43	14.47± 1.20	0.58± 0.09
98 mg/kg bw DLAE	8.20± 1.25	3.11± 0.32	11.22± 1.13	0.61± 0.06
49 mg/kg bw DLAE	7.58± 1.24	3.20± 0.38	13.60± 1.74	0.58± 0.10
24.5mg/kg bw DLAE	8.52± 1.36	3.70± 0.38	11.51± 1.46	0.56± 0.11

Values are given as mean ± SEM, no significant variations from the control group (n= 6). (DBAE) *D. maritima* bulb aqueous extracts; (DLAE) *D. maritima* leaves aqueous extract; (MDA) Malondialdehyde; (GSH) Reduced Glutathione.

Additionally, Table 4 equally shows the changes in lipid peroxidation and GSH content respectively in renal tissue. It is noted that the level of malondialdehyde (MDA) in DBAE and DLAE rats at all doses is comparable to that in control rats with (14.78 ± 1.68). Similarly, there was no discernible change in renal glutathione level when contrasted the treated rats to the untreated ones.

Effect of *D. maritima* aqueous extract on antioxidant enzyme activity changes in liver and kidney. Hepatic GPx values are presented in Table 5. Rats given doses of DBAE at 98 mg/kg showed non-significant higher levels of these antioxidant enzymes (24.26 ± 2.14) compared to controls. Furthermore, when compared to the normal rats, there were no appreciable differences in the GPx levels of the treated rats with DLAE aqueous extract. Parallel to these findings, liver GST levels in all treated animals were not substantially different from those of the control animals (Table 5).

The changes in renal GPx and GST activity levels are depicted in Table 5. Comparing the kidneys of rats given DBAE and DLAE aqueous extract daily to those of controls, no significant variations in glutathione peroxidase (GPx) activity was observed. Also, there was a non-statistically significant change in GST enzyme activity levels in all experimental groups compared to the control group.

TABLE 5
Antioxidant enzyme activity in liver and kidneys tissues of animals from different groups over a 4-week period.

Treatment doses	Liver enzymes		Kidney's enzymes	
	GPx activity [µmol GSH/mg prot]	GST activity [Nmol GST/ min/mg prot]	GPx activity [µmol GSH/mg de prot]	GST activity [Nmol GST/ min/ mg prot]
Control	9.47± 1.33	3.30± 0.35	14.78± 1.68	0.43± 0.03
98 mg/kg bw DBAE	8.85± 2.18	3.33± 0.29	13.72± 2.58	0.42± 0.04
49 mg/kg bw DBAE	8.81± 1.69	3.02± 0.27	12.52± 1.79	0.44± 0.03
24.5mg/kg bw DBAE	9.63± 1.78	3.72± 0.43	14.47± 1.20	0.39± 0.06
98 mg/kg bw DLAE	8.20± 1.25	3.11± 0.32	11.22± 1.13	0.34± 0.01
49 mg/kg bw DLAE	7.58± 1.24	3.20± 0.38	13.60± 1.74	0.38± 0.03
24.5mg/kg bw DLAE	8.52± 1.36	3.70± 0.38	11.51± 1.46	0.38± 0.04

Values are given as mean ± SEM, no significant variations from the control group (n= 6). (DBAE) *D. maritima* bulb aqueous extracts; (DLAE) *D. maritima* leaves aqueous extract; (GPx) Glutathione peroxidase; (GST) glutathione-S-transferase.

DISCUSSION AND CONCLUSIONS

The therapeutic potential of *Drimia* species has long been researched. However, even though these plants could offer a wide range of pharmacological advantages, a full investigation of the plant's toxicity is required. To assess the degree of exposure at which the activity of these plants becomes evident, toxicological tests of extracts from the aerial portion and bulb of the *D. maritima* plant were carried out in this work.

As part of our study on the effectiveness of *D. maritima*, we characterized the aqueous extracts produced from this plant. Qualitative phytochemical assays revealed a high phenolic compound content of the two extracts studied. The abundance of polyphenols in DBAE in the current study, is also confirmed by the phytochemical analysis of [11], [30]. The quantity of polyphenol discovered in DLAE matched that of [16] for the aqueous infusion of *D. maritima* leaves. Our findings also show that DLAE contains a considerable number of total flavonoids, phenolic compounds that are well-known for having antioxidant characteristics.

On the other hand, the anti-free radical activity of these two *D. maritima* extracts was calculated using the DPPH hydroxyl radical technique. This test depends on the reduction of this free radical into a stable product by the hydrogen-donating antioxidant molecules of the plant extract [31]. The results we obtained imply that both *D. maritima* extracts function as antioxidants, particularly DLAE because of its strong free radical scavenging ability. The presence of phenolic chemicals like flavonoids is considered to be connected to this antioxidant ability. These findings support prior research that demonstrates a link between plant extracts' antioxidant capacity and their phytochemical composition [32], [33]. The same antioxidant activity of *D. maritima* bulbs and leaves and *Drimia* genus was also demonstrated by [9], [13].

To evaluate an animal's general health and the safety of the product being used, it is vital to ascertain how its body weight has changed over time. In our experiment, the monitoring of weight growth revealed that the rats that received the various dosages of the aqueous extracts of *D. maritima*'s bulb and leaves (DBAE and DLAE, respectively) gained more body mass compared to the rats in the control group. On the one hand, a sufficient intake and absorption of food can account for this weight increase. These findings support those [34], [35], who showed that administration of an *Asparagaceae* family plant extract causes a considerable increase in body weight. On the other hand, earlier published research [36], [37] showed that these effects were linked to the ability of polyphenolic substances, flavonoids, and tannins contained in the plant. The abundance of *D. maritima* in these bioactive compounds has also been acknowledged by [10], showing the plant's advantageous anabolic action.

The hematopoietic system plays a predictive role in diseased status of both humans and animals, as one of the receiver profiles of several medications and xenobiotics [34]. Regarding the analysis of blood parameters, our findings showed no significant differences between rats from the treated groups and those from the control group, indicating a regular production of blood cells, which suggests that the extracts are not toxic to the blood system. These results are consistent with other studies [13] which claim that the administration of different extracts of *D. maritima* prevented hemolysis by boosting red blood cell resistance, proving the anti-hemolytic action of this plant. However, the number of WBCs only significantly increased at the high dosage of both DBAE and DLAE extracts. An excessive synthesis of the substances regulating the growth of leukocyte-producing stem cells could be the cause of this immune system response. Our results are in agreement with the research of [38] who report that this overproduction is related to the immunostimulatory activity of glycosides, and some phytochemicals present in plant extracts.

To predict the membrane integrity and function of hepatocytes following consumption of plant products, evaluation of the enzymatic activity of a few hepatic markers is crucial. Two transaminases in the hepatic serum with greater sensitivity are ALT and AST [39]. The present study reports nonsignificant changes in serum levels of these two enzymes, except for 98 mg/kg bw DBAE, for which a significant increase was observed. This may be attributed to changes in membrane permeability, which are often related to the presence of scillaren, the potent heteroside present in the bulb of *D. maritima*. In addition, the increase in ALP levels found in the liver at the bile canaliculi and marking the hepatobiliary transit state supports that scillarene acts on the activity of the enzyme Na^+/ K^+ ATPase, leading to a reduction in bile production in the ducts, which in turn reduces hepatic blood flow and leads to cholestasis [4], [6]. The liver synthesizes the essential plasma protein, albumin; fluctuation of the latter indicates a risk of liver disease [40]. This is clear from the study's results, which reveal that only the high dosage DBAE produced a modest drop in albumin levels while the other groups' levels were within the control group's reference range. More so, no significant changes were observed in total bilirubin, and lactate dehydrogenase, which also confirms the reduction of the risk of hepatobiliary damage and demonstrates the absence of hepatotoxicity of the extracts. These results are reinforced by the non-significance of the change in relative liver weight compared with the control group. Furthermore, *D. maritima* leaf extract did not cause significant changes in serum levels of liver enzymes, total bilirubin, albumin, and lactate dehydrogenase in treated rats compared to control rats, indicating that liver functions are not affected by DLAE administration, probably as a result of the flavonoids concentration as the major constituents of this extract. These outcomes are consistent with [41], which showed the hepatoprotective impact of five flavonoids.

The kidneys play an important role in the survival of the body by maintaining a constant tubular excretion and concentration of electrolytes in the blood [42]. For this purpose, renal functional status was assessed by measuring several indicators, including creatinine, urea, and uric acid. Data analysis showed that, in comparison to the control lot, rats given DBAE at a dosage of 98 mg/kg bw had significantly lower relative kidney weights. While DLAE had no noticeable effects, this shows that only high-level subacute exposure to DBAE acts on the kidneys and that DLAE does not harmful to the renal system. In addition, substantial increases in blood urea, creatinine and uric acid levels were observed only in high dose of DBAE extract, which may be related to the presence of glycosides. Similar findings were made by [7], [43] in which cardiac glycosides from *D. maritima* act on transmembrane proteins, increasing blood urea and uric acid levels. [44] indicate the abundance of bufadienolide cardiac glycosides in the *D. maritima* bulb that act as membrane enzyme antagonists. On the other hand, the absence of significant variations of these parameters in the other groups under treatment, indicates that the renal functions are not affected.

The body produces reactive oxygen species (ROS) primarily to aid in the detoxification process and the metabolism of cellular macromolecules. A surplus of these molecules directly contributes to the creation of oxidative damage [4]. In the present investigation, MDA levels in the liver and kidney did not change significantly after administration of DBAE and DLAE, at the different treatment doses. Thus, giving an indication of the lack of oxidative damage as it is the main biomarker of polyunsaturated fatty acid peroxidation in cell membranes [45]. In addition, the initial line of free radical detoxification, GSH content [46], was observed to be at an equivalent level to that of the control in all treatment groups. This justifies the antioxidant power of DBAE and DLAE extracts to prevent oxidative damage. These findings are in line with several research demonstrating the antioxidant properties of *Drimia* genus [4], [47].

On the other hand, enzymatic antioxidants are crucial for protecting cells from damaging compounds. In our research, the activity of two enzymes, GST and GPx, was investigated in the liver and kidney of untreated and treated rats. GST is a catalytic enzyme that works to lessen the toxicity of xenobiotics. The doses of DBAE and DLAE used in the experiment did not significantly alter this enzyme's activity. This outcome can be attributed to the antioxidant power of phenolic compounds found in both parts of *D. maritima*. Furthermore, [48] contends that these phenolic substances can improve and strengthen antioxidant enzymes by preventing the generation of ROS. In this regard, much recent research has proved the antioxidant capability of natural components derived from plants, including flavonoids and polyphenols [49], [50]. However, rats receiving high dose of DBAE showed a non-significant rise in the amount of glutathione peroxidase (GPx) hepatic, the enzyme that controls the level of ROS. During this time, all treated groups' kidneys showed insignificant amounts of GPx throughout the course of 4 weeks when compared to controls. These findings clearly demonstrate the ability of *D. maritima* extracts to promote and maintain cellular detoxification mechanisms.

In conclusion, according to the study's findings, *D. maritima* bulb aqueous extracts only have the potential to harm the liver and kidneys in very high levels. However, due to its numerous compounds having antioxidant characteristics, subacute oral administration of *D. maritima* leaf extract was deemed non-toxic at all doses tested in this study. This fosters the use of this plant's aqueous extracts and paves the

way for a wide range of environmental and health advantages.

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STUDY OF THE REPRODUCTION AND GROWTH OF *Sander Lucioperca* FROM BOUHAMDANE DAM, GUELMA, ALGERIA

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ABSTRACT

This study is based on the reproductive and growth biology of the specie *Sander Lucioperca* that is a freshwater fish, more responsive in the world and major in aquaculture. A total of 362 specimens of *S. lucioperca* were collected from the Bouhamdane dam in Guelma, by monthly sampling from november 2019 to october 2020. The sexes of specimens were determined macroscopically and the observed sex ratio was in favor of males during the whole cycle of study. This study was carried out to determine the gonado-somatic index (GSI), the liver somatic index (LSI) and the condition factor (K) show variations related to the reproductive cycle. For the study of age, 3 groups were identified for females and males. Size frequency distributions and growth parameters were analyzed using the ELEFAN1 method, using FISAT II software. According to Von Bertalanffy parameters, male pikeperch shows faster growth (L_∞=48.30cm, K=0.66 $year^{-1}$, Φ=3.18) than females (L_∞=46.20cm, K=0.57$year^{-1}$, Φ=3.08), which is rare in teleost fish. Weight growth varies between the two sexes, it is minorant in males and the combined sex (b<3) and isometric (b=3) in females.

KEYWORDS:
Sander Lucioperca, Reproduction, Growth, Von Bertalanffy parameters, Bouhamdane Dam

INTRODUCTION

In Algeria, the development of dam restocking is recent. It was around the 80s that the Ministry of Fisheries and Aquaculture decided to import pikeperch fry from Hungary as part of the evaluation and enhancement of water bodies to enrich the environment with ichthyological fauna and for the development of aquaculture [1, 2, 3].

The life history traits of pikeperch have been relatively well described, but mainly those of northern populations [4, 5]. The pikeperch is a large species, reaching maturity relatively late, with high relative fecundity and providing parental care. This set of characteristics classifies it as a species with a "periodic strategy" that maximize their fecundity in response to a stable (i.e., predictable) environment [6].

The study of the reproductive biology of pikeperch has been relatively well described but mainly those of populations located north of its range, namely the Baltic Sea and its appendices and Scandinavian continental waters [4, 7, 8, 9, 10, 11, 5, 12, 13]. Only a few studies have dealt with this aspect in Mediterranean regions such as those of [14, 15, 16, 17].

Various methods are now available to assess reproduction in fish, including microscopic gonad development, oocyte size frequency distribution, sex steroid measurement, and gonadal indices [18, 19]. Indeed, the gonadosomatic index (GSI), expressed as a percentage of gonadal mass relative to total body mass, is widely used as a simple measure of the extent of reproductive investment or gonadal development [20, 21].

Thus, the study of the sex ratio provides information on the proportion of male and female fish in a population, also indicating the predominance of sex in a population and thus giving basic information necessary for fish reproduction [22]. The condition factor was also estimated by directly measuring physiological parameters related to energy reserves such as tissue lipid content and reproductive status [23].

Reproduction is often the limiting factor in population dynamics. Indeed, in hydrobiology, growth parameters are an essential data for a good understanding of the biology and dynamics of animal populations, and a fortiori of fish [24, 25, 26, 27, 28, 29, 30, 31].

Our research work is based on the study of the reproduction, age and growth of this species in the Bouhamdane dam in Guelma. For many ichthyolo-

gists, data on fish age and growth are essential to understanding the life history traits of species and populations. Indeed, growth is one of the major processes in fish biology and is one of the key processes of length-structured models [32]. So far, no results concerning the biological characteristics of this specie have been obtained at the Bouhamdane dam.

The objectives of this study are to:

• provide information on the biology of the *Sander lucioperca* [33] populate the Bouhamdane Guelma dam and describe the reproductive characteristics of this species by analyzing the sex ratio, the gonado-somatic index, the liver somatic index, the condition factor, age and growth. The results of this study will help increase knowledge about the reproductive biology of *Sander lucioperca*, which is relevant for aquaculture management, as well as adequate supply.

• provide information on the population dynamics of *S. lucioperca* inhabiting the Bouhamdane dam in Geulma.

MATERIALS AND METHODS

Study area. Bouhamdane Dam (Hammam Debagh Dam) is located 23 km from the wilaya of Guelma since it is located 3 km upstream of the locality of Hammam Debagh, on the Oued Bouhamdane (Figure 1), takes its name from the area of thermal springs.

The body of water of the Bouhamdane dam (like that of Medjez Bégard) is a large lake, having its role to play in order to safeguard the environment, in addition to the purposes for which it was originally built, namely:

• Irrigation (34 hm^3) of the two perimeters Guelma-Bouchegouf (whose area extends over 13,000 ha) on the one hand and Drean-Besbès in the wilaya of El Tarf, on the other hand.

• Drinking water supply to the city of Guelma and surrounding centres (19 hm^3) [34].

Collection and sampling of specimens. A total of 362 individuals pikeperch (205 males and 157 females) were collected monthly from november 2019 to october 2020. After sampling, the specimens were afterwards transported immediately to the laboratory for being measured.

Body measurements. Total lengths (TL, cm) were measured using a one-meter measuring board graduated in cm, since the weight was evaluated by an electronic balance accuracy of 1g by considering the total weight (TW, g). All specimens were dissected to obtain eviscerated weight (EW, g), gonad weight (GW, g), liver weight (LW, g). Also, fish sexes (male or female) were determined by visual examination of the gonads and subsequently the mentioned parameters were recorded on a data collection sheet. The reproductive biology was determined by sex ratio (SR), gonad somatic index (GSI), liver-somatic index (LSI) and condition factor (K).

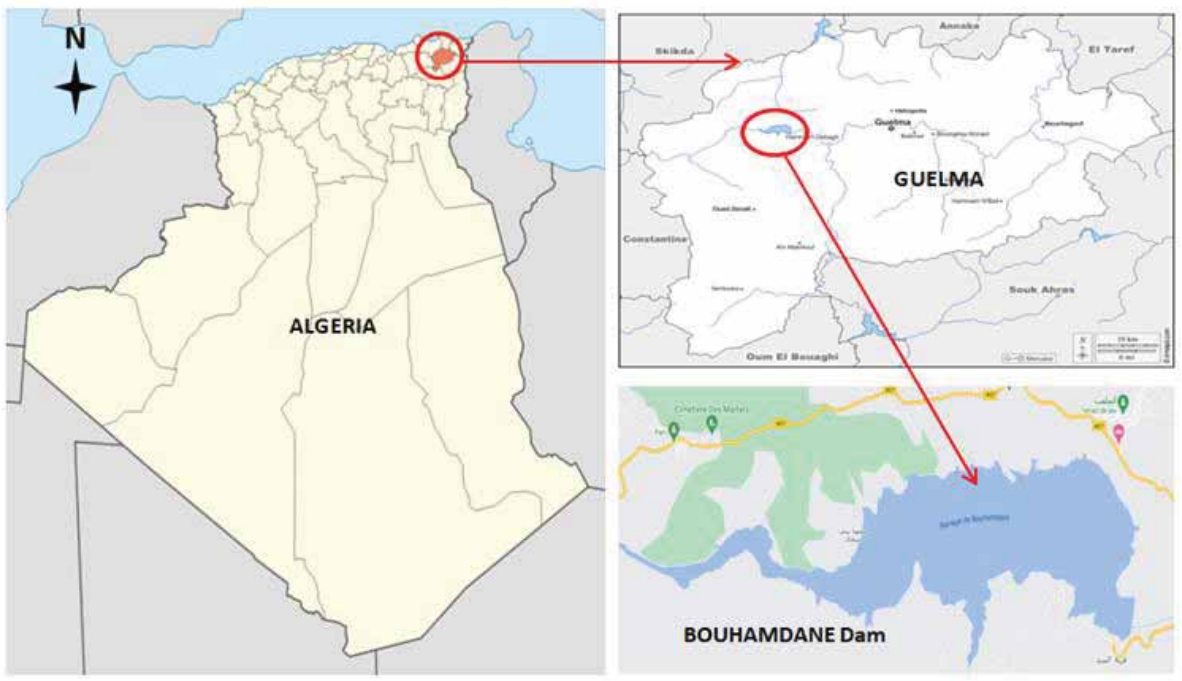

FIGURE 1
Location of the stady site, Bouhamdane Dam, Guelma (Google Mapp)

Sex ratio. In this study, we have adopted the definition of sex ratio as the proportion of males and females in the population [35].

$$proportion\ of\ males = (M/F + M) \times 100 \quad \text{(Eq.1)}$$

$$proportion\ of\ females = (F/F + M) \times 100$$

Where:

F: Number of females;

M: Number of males.

Gonadosomatic index (GSI). The GSI it is one of the parameters used in reproduction studies of fish. This parameter was estimated as the ratio of gonad weight to the eviscerate weight of the body, which can encrypt the growth of gonads during the reproductive cycle [36].

$$GSI = GW/EW \times 100 \quad \text{(Eq.2)}$$

Where:

GW: gonad weight (g);

EW: eviscerate weight (g).

Liver somatic index (LSI). The liver is a vital organ that plays a crucial role in the processes involved in the development of genital products [37]. The liver somatic index (LSI) was estimated as the ratio of liver weight to the eviscerate weight of the body [38].

$$LSI = (LW/EW) \times 100 \quad \text{(Eq.3)}$$

Where:

LW: liver weight (g);

EW: eviscerate weight (g).

Condition factor. The condition factor (k) of the experimental fish was estimated according to the following relation [39]:

$$K = (W/L^b) \times 100 \quad \text{(Eq.4)}$$

Where:

W: weight of fish (g);

L: total length of fish (cm);

b: slope of regression line.

Statistical analyses were performed using the Statistica, statistical program (software version 8.0). All results are subjected to ANOVA statistical analysis and student's test, in order to compare the average GSI and LSI.

Age and growth parameters. The fish's age was determined by removing 6 to 10 scales from the antero-medial part of the body above the lateral line of each specimen [40]. Scales were viewed by the stereo binocular microscope, and the scale reading was then examined twice by two independent readers.

Determination of growth parameters L_∞, K and t_0. In our study, the analysis of pikeperch growth parameters was carried out from the size frequency data of 362 specimens. The determination of the parameters L_∞ and K and the adjustment of the absolute growth curves are carried out by the ELEFEN I method using the FISAT II software [41].

Relative growth makes it possible to verify the existence of a correlation linking weight to fish size and to model the relationship.The length-weight relationship was calculated using the formula of [42,43]:

$$W = a \times TL^b \quad \text{(Eq.5)}$$

The formula was estimated by linear regression after logarithmic transformation of the data [44]:

$$\log W = \log a + b \times \log TL \quad \text{(Eq.6)}$$

Where:

W: body weight of fish in gram (g);

TL: total length (cm);

a: intercept;

b: slope of the regression line.

The parameter to was obtained from the empirical equation of [45]:

$$\log_{10}(-t_0) = -0.3922 - 0.2752 \times \log_{10} L\infty - 1.038 \times \log_{10} K \quad \text{(Eq.7)}$$

The growth performance index (Ø) was calculated to compare our results with those obtained in different regions. It was determined by the empirical equation of [46]:

$$\emptyset = \log K + 2 \times \log L\infty \quad \text{(Eq.8)}$$

The b value was analyzed by a Student's t-test, accordingly to the statistical equation reported by [47]. Following b values, the relation becomes isometric (b=3.0), minorant (b<3.0), and majorant (b>3.0) [48].

The weight growth equation (11) can be studied by the classical Von Bertalanffy model [49], by combining the Von Bertalanffy linear growth equation (9) and the height-weight relationship (10):

$$Lt = L\infty\left(1 - e^{-K(t-to)}\right) \quad \text{(Eq.9)}$$

$$Wt = a \times Lt^b \quad \text{(Eq.10)}$$

$$Wt = W\infty \quad \text{(Eq.11)}$$

With:

Wt: mass of the fish at time t (in years);

Lt: total length in cm at time t;

W_∞: asymptotic weight or maximum theoretical weight corresponding to Lt;

b: coefficient of the height-weight relationship or the slope of the line expressing the height -weight relationship in its logarithmic form.

t_0: theoretical age (year) that the fish would have had at size zero (Lt = 0)

K: growth coefficient.

RESULTS

The sex ratio according to the months. The values of the monthly sex ratio shows, a dominance of males over females throughout the year except during the two months September and November, when females are abundant with a significant percentage (60%, 70% respectively) (Figure 2).

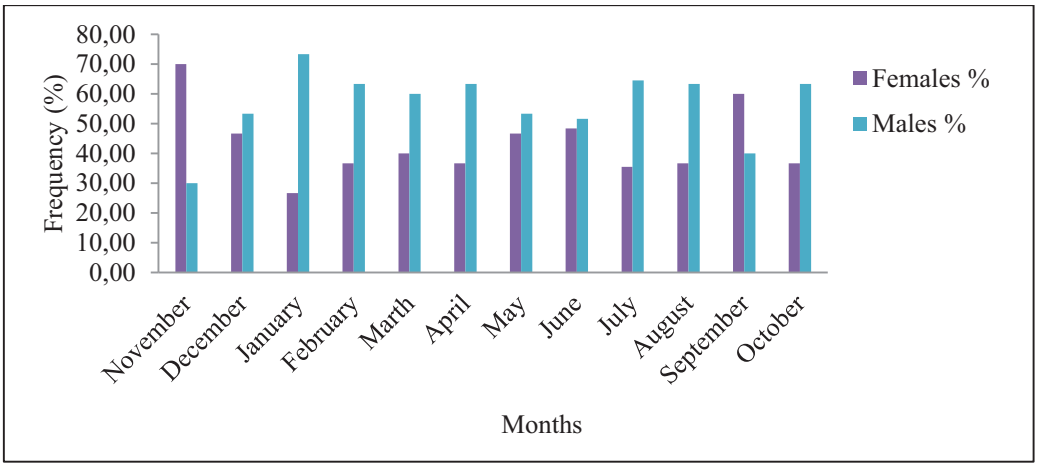

FIGURE 2
Monthly proportion of females and males for Sander lucioperca in Bouhamdane Dam during the study period (November 2019-October 2020)

There was no significant difference between the results obtained using the two methods (χ2calculated=19.15< χ2theoretical=19.67; p = 0.05) (Figure 2).

Gonadosomatic index (GSI). The monthly evolution of the GSI (Figure 3) has a similar trend for the total population and both sexes, except that, for males, the values are less important. Indeed, the maturation of genital products begins in December (females: 0.46±0.25%; males: 0.22±0.12%; population: 0.33±0.24%), and continues until March (females: 2.69±2.82%; males: 0.74±0.76%; both sexes combined: 1.52±2.07%). A drop in the GSI is observed in April to reach a low value in July for females and the total population (0.16±0.16%; 0.15±0.15%) and in August for males (0.04±0.01%). A resumption of this ratio is observed from July for females and the total population and September for males.

The ANOVA analysis shows a very highly significant monthly variation between the mean values of GSI of combined sexes, males and females (Fobs of combined sexes = 6.73, P≤0.001; Fobs of males = 6.89, P≤0.001and Fobs of females = 5.57, P≤0.001). According to student's t-test, there is a very highly significant difference between mean RGS values of the two sexes (t-test, d.f. = 360, p≤0.001) (Figure 3).

Liver somatic index (LSI). Minimum LSI values are observed in June for the total population, males and females (0.57±0.21%; 0.57±0.18%; 0.57±0.24%) respectively. From October, they increase gradually (Figure 4) until March for males (1.38±0.48%), females (2.03±0.64%) and the total population (1.64±0.61%) where the ratio reaches its maximum value (Figure 4).

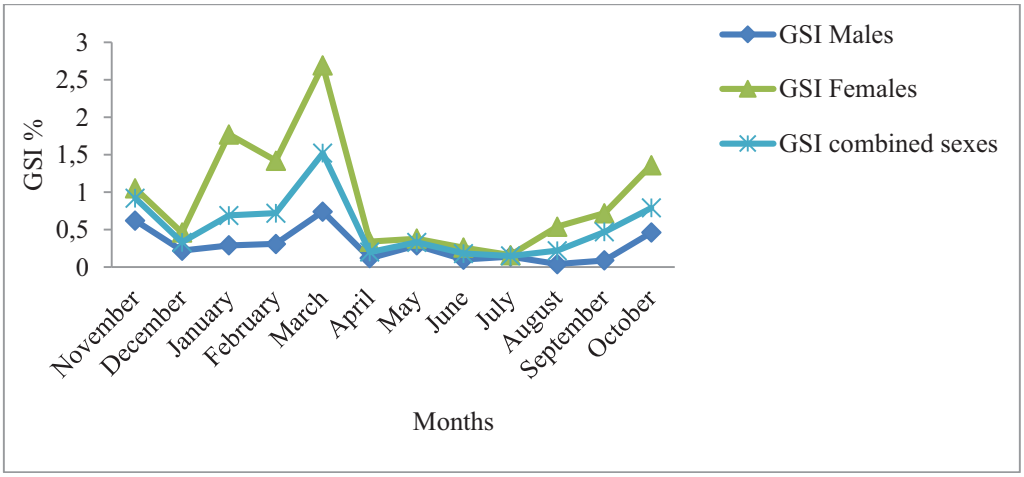

FIGURE 3
Gonadosomatic Index (GSI) for females and males of the Sander lucioperca in Bouhamdane dam during the study period (November 2019-October 2020)

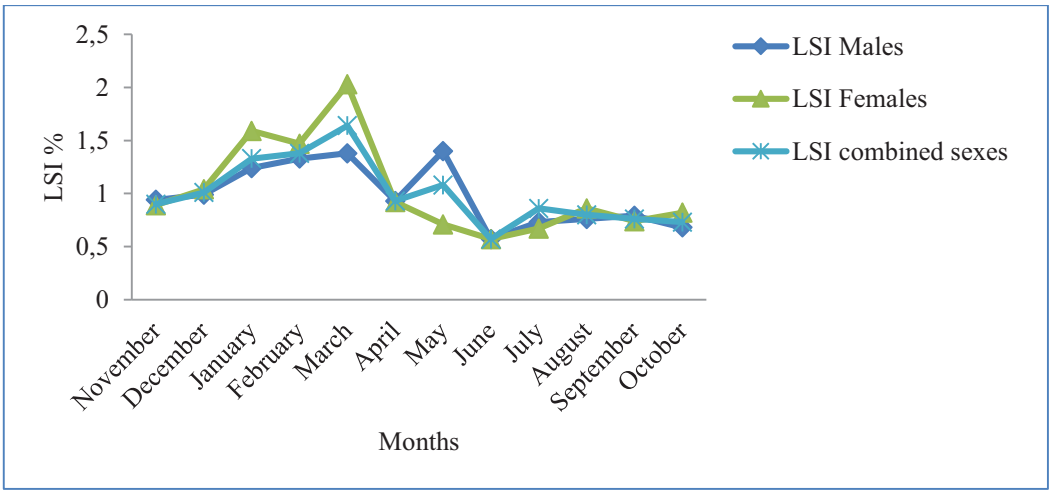

FIGURE 4
Liver Somatic Index (LSI) for females and males of the Sander lucioperca in Bouhamdane dam during the study period (November 2019-October 2020)

The ANOVA test shows a very highly significant monthty variation between the mean values of LSI of combined sexes, males and females (Fobs of combined sexes = 10.59, P≤0.001; Fobs of males = 3.74, P≤0.001; Fobs of females = 19.31, P≤0.001). with a threshold of 5% (Figure 04). The t-student test revealed a non-significant difference between the two sexes (t-test, d.f. = 360, p>0.05) (Figure 4).

Condition factor K. The values of the coefficient of condition K vary between 0.64±0.05% to 0.83±0.12% in females and between 0.65±0.04% and 0.79±0.03% in males. In winter, we note that the evolution of this index is more or less stable over time. In particular, during the laying period, this index reaches its maximum value in March-April for both sexes (K males =0.77±0.05%; K female =0.83±0.12%). A slight decrease in K is observed during the month of May for males (0.67±0.16%) and the months of May and August for females (0.73±0.07%; 0.66±0.04 respectively) (Figure 5).

Analysis of variance (ANOVA) showed a very highly significant monthly variation with a threshold of 5%, between the mean values of K (F obs of combined sexes = 15.24 ; p<0.001), (F obs of males = 7.77; p<0.001) and (F obs of females = 8.83 ; p<0.001) (Figure 5). There was no significant difference between the sexes (t-test, d.f.= 360, p>0.05).

Study of age and growth. For all individuals (n=362) of the S. lucioperca, the total weight extends between 106.5g and 719.8g (with an average value TW_{avg}=374.01g) while the total length varies between 25cm and 45.3cm (TL_{avg}=36.11cm).

Table 1 shows that it is the age class 2 that is the most dominant with 306 individuals and height values between 30.3 - 39.5cm, followed by age class 3 with 50 individuals, against only 6 individuals in age class 1(Table.1).

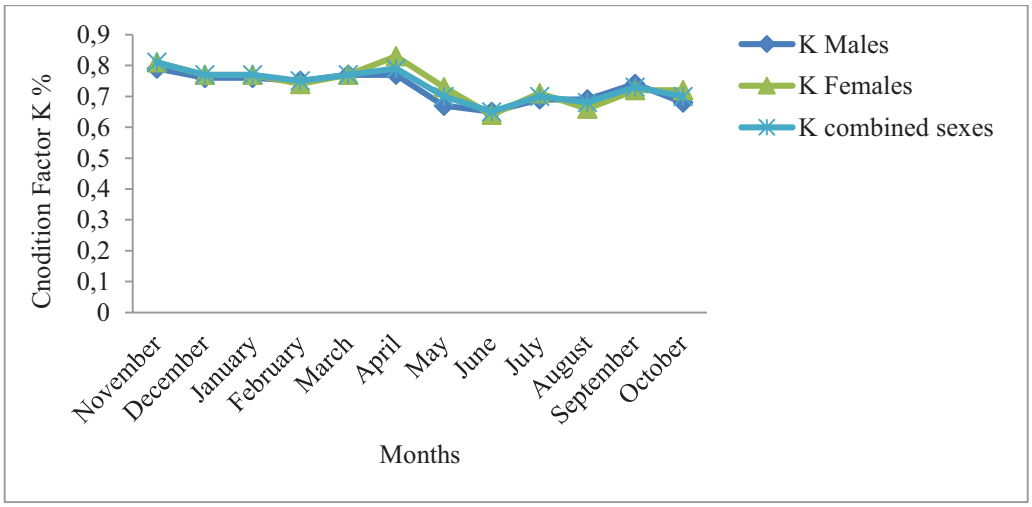

FIGURE 5
Monthly condition factor (K) for females and males of Sandes lucioperca in Bouhamdane Dam during the study period (November 2019-October 2020)

TABLE 1
Age-frequency distributions in total length and weight

Age groups			TL (cm)		TW (g)	
	N	F%	Min-Max	Mean ±SEM	Min-Max	Mean ±SEM
1	6	1.65	25-29.5		106.5-371.5	
2	306	84.53	30.3-39.5	36.11±0.036	195.8-588.6	374.01± 0.136
3	50	13.81	40-45.3		459-678.6	

N: sample number; F: frequency; TL: total length (cm); TW: total weight (g); min: minimum; Max: maximum; SEM: Standard error of the mean

Linear Relative Growth and Absolute Weight Growth. The linear growth parameters of the Von Bertalanffy model [49] and the index of growth performance (Φ′) were estimated for males, females and both combined sexes.

The asymptotic lengths calculated are perfectly in line with the maximum lengths observed (Table 2). The asymptotic lengths (L_∞) obtained in the total population (L_∞=48.30cm), females (L_∞=46.20cm) and males (L_∞=48.30cm) are higher than the maximum lengths which are respectively L_{max}=45.3cm for the total population, L_{max}=44.5cm for females and L_{max}=45.3cm for males.

The value of the asymptomatic length (L_∞=48.30cm) of males is higher than that of females (L_∞=46.20cm), a difference of 2.10cm, also that the K value for males is higher than that of females. The value of the growth performance index (Φ′) calculated for a male is higher than for females (Table 2, Table 3).

Length-weight relationship. The estimated annual values of b (regression slopes) are less than or equal to 3, so Student's t-test gives us higher values; threshold corresponding to a degree of safety of 95% (t-test, $p > 0.05$).

This would indicate that the *Sander lucioperca* population is characterized by a minor allometry between total weight and length in males (b=2.852; r^2=0.759) and total population (b=2.805; r^2=0.711) (weight grows less rapidly than the cube of length) (Table 4).

For females, the coefficient b of the length /eviscerated weight relationship is equal to 3 at the risk of error 5% (test-t, $p < 0.05$), it would be an isometric allometry (b=2.978; r^2=0.615) (length grows at the same rate as weight).

The equations of total length (TL) and eviscerated weight (EW) show positive correlations of all pikeperch individuals (Table.5).

DISCUSSION

Current knowledge of the biology of *Sander lucioperca* (mainly reproduction) is considered insufficient to improve stock assessment [16]. In the Bouhamdane dam, analysis of the population structure of *S. lucioperca* showed a predominance of males (male population) throughout the sampling period except in September and November when females are dominant. The sex rate is (56.63%) so males are more numerous than females (43.37%). Similar results are obtained by [17] in the Ghrib dam in Ain Defla which shows a sex ratio still in favor of pikeperch males.

Regarding the study of the sex ratio as a function of size, we find that males are dominant up to the size of 43cm, beyond this size, the proportions of females are more dominant, up to the maximum size. Similar results were obtained by [50, 16] and [51] in reservoirs in France, by [14] in Tunisia and by [17] in the Ghrib dam in Ain Defla.

TABLE 2
Parameters of Von Bertalanffy of S. lucioperca according to sexes (males, females and both)

Sex	L_∞(cm)	W_∞(g)	K (yr^{-1})	t_0 (yr)	Φ′
Males	48.30	744.52	0.66	-0.21	3.18
Females	46.20	773.80	0.57	-0.25	3.08
Combined	48.30	816.11	0.63	-0.22	3.16

TABLE 3
Von Bertalanffy equation of S. lucioperca according to sexes (males, females and both)

Sex	Von Bertalanffy Equation	
	Linear growth	Weight growth
Males	$TL = 48.30\ [1\text{-}e^{-0.66\ (t\ +\ 0.21)}]$	$TW = 744.52\ [1\text{-}e^{-0.66\ (t+0.21)}]^{2.535}$
Females	$TL = 46.20\ [1\text{-}e^{-0.57\ (t\ +\ 0.25)}]$	$TW = 773.80\ [1\text{-}e^{-0.57\ (t+0.25)}]^{3.083}$
Combined	$TL = 48.30\ [1\text{-}e^{-0.63\ (t\ +\ 0.22)}]$	$TW = 816.11\ [1\text{-}e^{-0.63\ (t+0.22)}]^{2.750}$

TABLE 4
length-eviscerated weight relationship parameters of S. luciperca from the Bouhamdane dam

	N	*a*	*b*	r^2	t_{cal}	Significance	Allometry
Males	205	0.0120	2.852	0.759	16.33	+	Minorant
Females	157	0.0078	2.978	0.615	15.66	+	Isometric
combined	362	0.0146	2.805	0.711	29.76	+	Minorant

N: number of sample; a: intercept; b: slope; r^2: coefficient of determination; t_{cal}: t-test calculated; (+ ; -) significance at 95% confidence level of t-test.

TABLE 5
length- eviscerated weight relationship of S. lucioperca from the Bouhamdane dam

	$EW = a \times TL^b$	Log EW = Log a +b × Log TL
Males	$EW = 0.0120 \times TL^{2.852}$	Log EW = -1.915 + 2.8529 × Log TL
Females	$EW = 0.0078 \times TL^{2.978}$	Log EW = -2.104 + 2.9785× Log TL
combined	$EW = 0.0146 \times TL^{2.805}$	Log EW = -1.836 + 2.8051× Log TL

Knowledge of the laying period and its duration is important for understanding population dynamics, particularly in the larval and juvenile stages. Authors have determined the maximum laying period of *S. lucioperca* [33] in European, Asian and North African reservoirs based on the abundance of eggs and larvae, the histology of the ovaries of the females of *S. lucioperca* or the analysis of the GSI (gonado-somatic index) [50, 10, 14, 52, 53, 11, 5, 54].

The ratio between gonad weight and body weight makes it possible to evaluate investment in reproduction [55, 56]. GSI is a true coefficient of maturation of the gonads. Its increase coincides with gametogenesis, while its decrease indicates active egg-laying [57].

During the sexual cycle, we have noticed that the weight changes of the ovaries and testicles are synchronous. The percentage of GSI of females is greater than that of males due to the large size of the ovaries. The gonado-somatic index of *S. lucioperca* gradually increases from December, marking the beginning of the maturation of the gonads. It reaches its peak in March, which indicates the final phase of vitelloggenesis (maturation and hydration of oocytes with spermiation) whose maximum value is detected in females (2.69±2.82) and males (0.74±0.76) in March.

Egg-laying and emissions begin resulting in a drop in GSI in April for both sexes. From this last month, the GSI remains low indicating the staggering of sexual rest until the following September for the resumption of another annual cycle. Monitoring the monthly variations of the GSI allowed us to know that the breeding period of the pikeperch of the Bouhamdane dam is seasonal and occurs in spring, ranging from March to April. This situation is common to all reservoirs in Europe [4, 50, 8, 9, 10, 11, 5, 12, 13] and in North African reservoirs [14, 53, 17].

The monthly mean values of the LSI show significant fluctuations related to the reproductive cycle of the species. The observation of the temporal evolution of the LSI of pikeperch shows phases of hepatic synthesis and phases of hepatic lipid consumption in the same way in both sexes. The maximum LSI value is recorded in March (2.03±0.64 for females and 1.38±0.45 for males). This value gradually decreases to its minimum in June and extends until July in females, which synchronizes perfectly with the period of sexual rest in both sexes. From September, this ratio increases gradually and suggests a taking of the storage of liver reserves for a new sexual cycle.

The monthly variation curves of the liver somatic index and the gonado-somatic index show that the maximum of the GSI coincides with that of the LSI. Our results corroborate those of [17] in the Gherib dam. This is explained by the fact that *S. lucioperca* is a fatty fish. It stores energy reserves mainly in the muscles, in the perivisceral mesentera and under the skin [58]. These reserves then pass into the liver and gonads to ensure energy needs during the reproductive season, hence the decrease in the condition factor during the reproductive period. Indeed, ovarian development is accompanied by maximum energy expenditure [59].

The seasonal variations in the coefficient of condition K of male and female pikeperch show a seasonal cycle, where overweight records high values in autumn, spring and winter and minimal in summer. [60] suggest that gametes are produced during autumn, winter and spring. The increased condition factor in March and April (laying period) this could be due to the development of the gonads. A similar trend has been observed in other pikeperch species [16]. On the other hand, in the lake of the Ghrib dam in Ain Defla, the K weight index reaches its minimum in spring [17]. *S. lucioperca* is affected by sexual activity, presence, density, variety of prey (species, sizes, nutritional quality) and physico-chemical conditions of the environment that would strongly influence the condition of fish and their ability to grow, reproduce and survive [17]. Some fish species may also be sensitive to changes in water temperature: a change in the surrounding temperature at critical periods of development can alter the proportion of males and females in a population [61]. The initiation of the reproductive cycle in pikeperch takes place under decreasing temperature [16]. Egg-

laying occurs when water temperatures reach 10-14°C in Finland [62] and 14-15°C in Tunisia [63], but can occur in a wider temperature range of 4.5 to 26°C [64]. Temperature is a major factor in triggering reproduction in Percidae [65] and promotes full gonadal maturation in both pikeperch sexes [66].

According to the work carried out on the reproductive aspect, the laying period is in March-April when the water reaches about 12°C which is within the values usually described for pikeperch, namely between March and May at temperatures between 8 and 15°C [4]. Meanwhile, [5], report that pikeperch generally breeds between April and June. In Algeria the breeding period of this species is between March and May [1]. While it takes place from late February to the first half of March in the Sidi Salem dam in Tunisia [15]. However, its thermal optimum of reproduction is 14-18°C [67]. At maturity (2-3 years for males and 3-4 years for females, which corresponds respectively to 33-37cm and 40-45cm; [16]. Males showed reduced mobility compared to females, or even completely stationary phases, mainly between March and April. This behaviour corresponds to the making and guarding of the nest by the male [68, 69, 5].

A pikeperch grows fast enough, a one-year-old pikeperch can measure up to 25cm if food is plentiful. At two years old it can reach 35 to 40cm and becomes able to reproduce but most of the time it is at three years. It then grows about 10cm per year but this is an average that varies according to biotopes and individuals. The average maximum height is just over 1m for 15 to 20kg [70].

The maximum age determined in the Bouhamdane dam (Geulma, Algeria) does not exceed 3 years in males than in females, because according to the DPRH in Guelma (comm per) this dam has undergone a seeding operation for three years. The number of specimens suitable for the determination of age classes was 362 (157 females and 205 males), with total sizes ranging from 25cm to 45.3cm, with a dominance of the 2-year-old group. On the other hand, our results show total lengths (TL at the age of 3 years) lower than those revealed in France, by [71] in southern rivers (TL_{max}=51.5cm). In Algeria, similar results were obtained in the Ghrib dam by [72] (TL_{max}=44.5cm) in the same species.

[71], in rivers of southern France, reported that the age of S. lucioperca ranged from 1 to 5 years. In the Ghrib dam, [72] indicated that the lifespan in both sexes was 1 to 9 years, were recorded for sizes ranging from 21-88.2cm.

[16], in Fumemorte (France) reported that the maximum age was 7 years. These differences in age distribution may be due to fish activity, diet and ecological characteristics of lakes [73].

The asymptotic length of the total population (L_∞=48.30cm) is greater than the maximum total length observed (TL=45.30cm). It is the same for that of females (L_∞=46.20cm) and males (L_∞=48.30cm) which are greater than the maximum total length observed, respectively 44.50cm and 45.30cm. Comparisons between Von Bertalanffy growth parameters for a number of *S. lucioperca* are summarized in (Table 6). The estimation of growth parameters by the Von Bertalanffy model varies depending on the region and the method used.

In this study, L_∞ values cannot be discussed from a 3 year-old population t_o 5 or 6 year-olds. Regarding the growth rate (K) in pikeperch, our data (K=0.63 $years^{-1}$) are close to those reported in Tunisia, by [14] (K=0.53 $years^{-1}$).

However, our results are higher than those recorded in Germany by [74] (K=0.20 $years^{-1}$), and in Algeria (Ghrib dam) by [73] (K=0.13 $years^{-1}$).

In addition, the growth performance index (Ø') shows high values (3.16) for the population as a whole; 3.08 for females and 3.18 for males, it is close to that obtained in Germany by [74] (Φ'=3.09) and in Turkey by [77] (Φ'=3.04), but it is less lower than those obtained in Tunisia by [14] (Φ'=3.36) and in Algeria by [72] (Φ'=3.31).

According to [80], the results of the population study are reliable only if the values of the growth performance index are between 2.65 and 3.32. Thus, the phi-prime (Φ') values calculated in this study are in the range of estimates since it is greater than 3.

TABLE 6
Von Bertalanffy parameters (L_∞, K, t_0 and Ø') of Sander lucioperca at different localities

Locality	L_∞(cm)	K	t_0	Ø’	Author
Germany	86.00	0.2	-0.63	3.09	[74]
Tunisia	66.00	0.53	-0.24	3.36	[14]
German coastal waters, Baltic Sea	141.3	0.085	-1.354	2.88	[75]
Turkey (Lake Eğirdir)	95.4	-0.084	1.563	2.88	[76]
Turkey (Lake Eğirdir)	156.95	-0.045	2.622	3.04	[77]
Iranian Coastal Waters, Caspian Sea	55.05	0.15	-2.59	2.65	[78]
Southern Finland	69.56	0.10	-0.12	2.63	[79]
French Reservoir	96.8	0.03	-4.38	2.44	[51]
Algeria (Ghrib Dam)	125.72	0.13	-0.89	3.31	[72]
Algeria (Bouhamdane Dam)	48.30	0.63	-0.22	3.16	Present study

This heralds rapid growth of the local population and indicates good sampling and uniform selection of *S. lucioperca* specimens at the Bouhamdane dam. The differences between the values of the growth performance index estimated by the authors can be explained by: sampling data, sample sizes, at the sampling period (seasonal variation), hydroclimatic and environmental conditions dominant [81].

The difference in growth parameters across regions is likely explained by differences in environmental conditions, such as water, temperature, quantity and abundance of food [82]. According to [83], the growth rate is influenced by variations in salinity and water temperature, as well as fish feeding habits.

[84] was shown that a salinity of 2 to 5g.L^{-1} significantly increased the growth of pikeperch. Water temperature has a direct impact on the physiology of fish fauna growth and reproduction [85] in [86].

The change in growth rate between males and females is influenced by sexual maturity. The reversal of the growth rate between males and females in our study (K = 0.66 and 0.57 respectively and Φ'=3.18 and 3.08) at the limit of sexual maturity can be explained by the difference in metabolism between the two sexes such as the difference in oxygen consumption [87] and/or the difference in the level of excess energy between reproduction and somatic growth [88].

The growth of pikeperch depends on the richness of the environment in food and abiotic factors, the optimum of its growth is located at a temperature ranging from 20 to 28°C. The growth of pikeperch is slowed around September because of the pre-maturation of the gonads [16]. According to a study done in Tunisia by [89], it has been shown that females show a higher growth in length than that of males. This importance is even higher with age. This difference in growth between the two sexes is that females feed more than males, because their needs are high in energy essential for the proper functioning of their metabolic and reproductive function.

The difference in growth between the two sexes allows females to reach larger sizes than males for the same ages [90]. Growth was faster in females [91, 92] or males [93]. However, there are cases where there is no significant difference in growth between the two sexes [94].

Fitting the growth parameters to the growth model of Von Bertalanffy [49] shows that the values of asymptotic weights for females (P_∞=773.80g) are higher than those for males (P_∞=744.52g). According to some authors, females grow faster than males [85, 86].

Our results indicate that pikeperch exhibits rapid growth of young individuals. According to Von [49], the growth of animals is very rapid in the young, it becomes slower and slower with age. These results are consistent with several authors for various populations of both marine and freshwater fish [95, 96, 97, 81].

Regarding the relative growth or lenght/total weight relationship in *Sander lucioperca* in the Bouhamdane dam is of the minor allometry type in the total population with b=2.75, in males b=2.53, indicating an increase in the size of individuals faster than that of weight; However, this same relationship shows isometry in females with b=3.08.

Values of b equal to 3 indicate that the fish is developing isometrically, which means that the lenght and weight increase proportionally. The comparison of b values with those obtained by other authors is summarized in (Table 7); we note differences from one region to another.

Both parameters of the lenght/weight relationship are very sensitive to size composition, sampling period, sex and maturity [98, 99].

TABLE 7
Parameters for length-weight relationship of Sander lucioperca at different localities (a: intercept; b: allometric coefficient)

Author	location	a	b	Allometry
[101]	Finland (Lake Vanajanselkä)	0.0076	3.04	Isometric
[100]	Romania (Lake Razim)	0.0058	3.14	Majorant
[102]	France (Castillon reservoir)	4.9×10^{-6}	3.07	Isometric
[103]	France (Treignac reservoir)	4.87×10^{-6}	3.05	Isometric
[107]	Turkey (Hirfanli Dam Lake)	10×10^{-6}	3.07	Majorant
[76]	Turkey (Lake Eğirdir)	0.006	3.148	Majorant
[77]	Turkey (Lake Eğirdir)	0.022	2.742	Minorant
[78]	Iranian Coastal Waters, Caspian Sea	-0.0206	2.85	Minorant
[79]	Southern Finland	0.00217	3.371	Majorant
[63]	Tunisian reservoirs	5×10^{-6}	3.06	Majorant
[51]	French Reservoir	1.91×10^{-9}	3.25	Majorant
[102]	Turkey (Lake Marmara)	0.0091	2.996	Isometric
[72]	Algeria (ghrib dam)	0.0033	3.237	Majorant
Present study	Algeria (Bouhamdane Dam)	0.0188	2.7504	Minorant

Sander lucioperca in the Bouhamdane dam shows a minor allometry, on the other hand, In Romania (Lake Razim), [100] (b=3.14), and in Algerians (Ghrib dam), [72] (b=3.23) considered respectively, that the relative growth is higher in the total population. In Finland (Lake Vanajanselkä) [101], in France (Castillon reservoir) [102] and (Treignac reservoir) [103], in Turkey (Lake Marmara) [104]) reported that weight increases at the same rate as sander size.

These variations in the type of allometry from one region to another could be attributed to differences in age, maturity, sex, environmental conditions [40] and parasitic pathologies are all factors that can affect the value of b [42, 105, 40, 106].

The study of the length-weight relationship generally meets two objectives in fisheries: the determination of the weight of individuals whose size we know or vice versa and the description of shapes, overweight and its variations during growth.

These parameters are of practical interest in the problems of rational exploitation of animal populations where it is important, to obtain maximum yield, to know how to translate lenght into weight or weight into size and to have an easily calculable parameter that characterizes the overweight of specimens and its evolution during the year or their life [81].

CONCLUSION

In light of the results obtained from the study of reproductive biology and population growth of *Sander lucioperca* populating the Bouhamdane dam (wilaya of Guelma) during a sampling year (from November 2019 to October 2020) it can be concluded that:

- The Sander lucioperca is an annual group-synchronous layer.
- The exploitable stock of *Sander lucioperca* contains more males than females.
- Analysis of the gonado-somatic index made it possible to determine the reproductive period. According to our results, the peak of the GSI is recorded in March.
- The analysis of the monthly evolution of LSI in both sexes shows that it evolves in parallel with GSI, this allows us to inspire that *Sander lucioperca* is a fatty fish and that it can have the necessary reserves for its sexual maturation from the muscles. The liver intervenes only in the transformation of these reserves towards the gonads during the breeding period.
- The condition factor is used to quantify the overweight of the fish; thus, a high K means a better condition of the fish. In our study, condition factor K means that both sexes have a similar development of overweight throughout the year.
- In this study, the value of the allometric coefficient of the length-weight relationship indicates a decrease in allometry for males and both sexes combined (b=2.535 and b=2.7504 respectively). Whereas, females have isometric allometry (b=3.083). In this case, the linear growth of *S. lucoperca* in the Bouhamdane Dam is faster than the weight growth in males and at the same rate in females.

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PHYSICOCHEMICAL, BIOCHEMICAL AND MICROBIOLOGICAL QUALITY OF DRIED AND SALTED CAMEL MEAT (KADID) FROM THE SOUTHWESTERN REGIONS OF ALGERIA

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ABSTRACT

This study aims to assess the physicochemical, biochemical, and microbiological quality of dried and salted camel meat, a traditional product of the inhabitants of southern Algeria.

A total of 8 test samples of dried and salted camel meat from three regions in southwestern Algeria, against two other control samples (i.e 10 samples in total), were subjected to a series of analyses for some physicochemical and biochemical parameters (pH, sodium chloride content, total solid content, moisture content, ash content, water-holding capacity, protein content, fat content, total sugar content, and detection of antibiotic residues), and microbiological parameters (TAMF, fungal flora, total and fecal coliforms, fungal flora, SRC, *Bacillus* spp, and pathogenic bacteria such as coagulase-positive staphylococci, *Listeria monocytogenes*, *Pseudomonas* spp, and *Salmonella* spp), following official and reference methods.

The physicochemical results showed that the pH and titratable acidity of the dried and salted camel meat samples were acidic with values ranging from 5,14 to 5,72; and 3g/L to 3,63g/L, respectively. The analyzed samples had sodium chloride content ranging from 0,09g/L to 3,31g/L, (6,46% to 25,34%) moisture content, (3,86% to 38,58%) ash content, (162,5% to 267,7%) water-holding capacity, against (0,15g/L; 0,12g/L); (25,34%; 76,75%); (5,78%; 4,73%); (162,52%; 99,33%) for the control samples, which are laboratory-prepared dried camel meat, and fresh camel meat sold in Bechar city, respectively. However, biochemical parameters showed a protein content ranging from 21,7% to 36%, (4,9% to 23,3%) fat content, and (20mg/L to 76,51mg/L) total sugar content, with no antibiotic residues.

The microbiological results showed a TAMF load ranging from 4,02 to 6,46 Log_{10} CFU/g, and 2,48 to 5,21 Log_{10} CFU/g for total coliforms, where 33% of the samples were contaminated with fecal coliforms. All samples analyzed were free of pathogenic bacteria, namely coagulase-positive staphylococci, *Listeria monocytogenes*, *Pseudomonas* spp, and *Salmonella* spp. *Bacillus cereus* was detected in one sample (C), while the isolated CoNS species were identified as follows : *S. saprophyticus*, and *S. epidermidis*.

From previous studies revealing various recipes for preparing dried and salted meat in the southwestern regions studied, this meat by-product presents very interesting physicochemical, biochemical, and hygienic characteristics at the nutritional level compared to fresh meat, which not only contributes to its preservation but also improves its sensory quality.

KEYWORDS:
Camelus dromedarius, Dried and salted camel meat (Kadid), microbiological quality, physicochemical and biochemical parameters, Southwestern Algeria

INTRODUCTION

Camels (*Camelus dromedarius*) are one of the most important sources of meat in the desert since they can withstand extreme temperatures and food scarcity conditions, they are also, a means of transportation and have been used as medicines for diverse ailments since ancient times [1-3]. To this day, meat has remained an important source of nutrients in Algeria. It is an excellent food whose consumption is only hindered by its high cost (beef and lamb meat), unlike camel meat which is less expensive compared to other red meats, allowing low-income families to have access to red meat at a lower price [4]. In arid and semi-arid regions of the world, camel meat is a very important food source for Sudan, Somalia, and Mauritania [5]. In the southern regions of Algeria, camel meat is considered the most consumed compared to other types such as beef and lamb [6-8].

Due to its moisture content and proteins of high biological value [9], camel meat is an essential food for a balanced food ration for the population of

southern Algeria [10]. Camel meat is healthy and nutritious as it contains low fat and cholesterol contents (especially young camels) as well as being a good source of minerals and is used to remedy various diseases [2, 3, 10-12]. For this, camel meat has become a popular alternative to other red meats [2]. However, the nutritional quality of meat constitutes a favorable environment for microbial development [13], as the hygienic quality of meat depends, on the one hand, on contamination through operators' hands, tools, and work plans during slaughtering and cutting operations, and on the other hand, the development and growth of microbial contaminants during conservation, storage, and distribution [14, 15]. To remedy this constraint, several generation-to-generation preservation methods have been developed [16].

The use of traditional methods of preserving meat dates back to prehistoric times, where salting, drying, etc., were done to preserve its microbiological quality by slowing down the proliferation speed of microbial contaminants and improving the sensory and nutritional qualities of meat by excluding intrinsic and extrinsic alteration mechanisms [17]. Traditional meat products are highly sensory quality foods, usually with high nutritional value, produced on a small scale, using ingredients and procedures from ancient times [18, 19].

Several research studies have been conducted on the quality of camel meat at the national and international levels. However, dried and salted camel meat (kadid, also known as El Guedid) as a by-product has not been broadly reviewed to date. The study aims to evaluate the physicochemical, biochemical, and microbiological characteristics of dried and salted camel meat samples from three different regions in southwestern Algeria (Bechar, Beni Abbes, and Tindouf).

MATERIALS AND METHODS

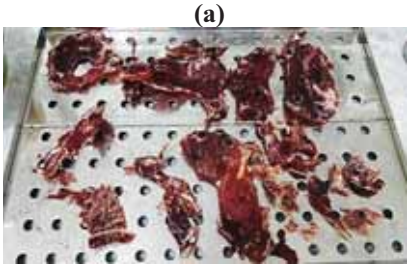
The study aims to evaluate the physicochemical, biochemical, and microbiological characteristics of some samples of dried and salted camel meat (Kadid) from the Sahraoui camel breed, prepared using traditional methods in the southwestern regions of Algeria, as described by Benyagoub and Bessadet [19], against two control samples. The different parameters analyzed were performed at Mohammed Tahri University of Bechar (Algeria).

Sampling. A total of ten (10) samples were analyzed (Table 1, Figure 1), of which eight (8) samples were dried and salted camel meat against two control samples (fresh camel meat 'TF' as unprocessed meat, and dried camel meat 'TS' prepared in the laboratory).

Sample preparation for physicochemical and microbiological analyses. Before performing the various analyses, the samples were ground using an electric shopper, and then the ground sample of dried and salted camel meat obtained was placed in a sterile stomacher bag (Figure 2). According to national regulations [20, 21], 0,1% peptone-salt solution was used as a diluent in the preparation of the stock suspension and decimal dilutions of the samples.

Physicochemical analysis. The physicochemical parameters analyzed are given in Table 2.

(a)



(b)

FIGURE 1
Dried and salted camel meat (Original, 2023).
(a): Dried and salted camel meat (test samples);
(b): Control sample of dried camel meat prepared in the laboratory (TS).

FIGURE 2
Ground sample of dried and salted camel meat (Original, 2023).

Bacteriological analysis. The bacteriological parameters analyzed are given in Table 3.

Bacterial identification. The isolated bacteria from selective media were subjected to several tests for identification according to standard microbiological methods as described by Benyagoub et al. [10]; Tille [41]; and Benyagoub et al. [42].

Interpretation of physicochemical and bacteriological analysis results. Since there are no thresholds related to physicochemical parameters in national regulations, we compared the results obtained with those reported by other scientific studies. Dried and salted camel meat and fresh meat samples were assessed based on the maximum limits for 'mechanically separated meat' and 'meat preparation' products given by the national regulation [43].

Detection of antibiotic residues. The agar-well diffusion method was used for the detection of antibiotic residues. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as reference bacterial strains on Mueller Hinton agar medium (MHA), where three techniques were used, first, the wells made on MHA were filled with 50µL of stock solution of dried camel meat, while the second was done by placing a piece of dried meat on MH agar incubated with the reference strains cited above [44, 45], and third, antibiotic test kit based on the colloidal gold immunochromatography technology used for detecting antibiotic residues from tetracyclines, beta-lactams, includind cephalexin.

TABLE 1
Sampling and preparation history of dried and salted camel meat.

Samples	Kadid preparation area	Date of preparation	Drying time
A	Lahdeb (Bechar province)	28 Oct – 6 Nov 2022 at ambient temperature	09 days
B	Igli (Beni Abbes province)	4 – 19 Dec 2022 at ambient temperature	15 days
C	Tindouf province	22 – 31 Dec 2022 at ambient temperature	09 days
D	Bechar El-Djadid (Bechar province)	5 – 17 Jan 2023 at ambient temperature	12 days
E	Timoudi (Beni Abbes province)	29 Nov – 8 Dec 2022 at ambient temperature	09 days
F	Abadla (Bechar province)	10 – 19 Jan 2023 at ambient temperature	09 days
G	Ouakda (Bechar province)	10 – 19 Nov 2022 at ambient temperature	09 days
H	Blue area (Bechar province)	27 Nov – 9 Dec 2022 at ambient temperature	12 days
TS	Dried camel meat prepared in the laboratory as a control sample	20 – 23 Feb 2023 at 25°C	03 days
TF	Fresh camel meat as a control sample (Bechar El Djadid market)	/	/

TS: Control sample of dried camel meat prepared in the laboratory; TF: Fresh camel meat as control sample; Source: Own study.

TABLE 2
Physicochemical analysis of camel meat.

Physicochemical parameters	Analytical method or device used	References
pH (°C)	pH meter	JORA n.23 [22]
Titratable acidity	Titrimetry method (Indicator method)	AOAC [23]
Sodium chloride content (g/L)	Argentometric titration method (Mohr's method)	Soderberg [24]
Water-holding capacity of meat (WHC %)	The gravimetric method with application of external force (Centrifuge at 6500rpm for 20min)	Honikel [25] Huff-Lonergan [26]
Moisture content (%) Total solid content (TSC %)	Direct drying method at 105°C (Thermogravimetric approach)	JORA n.1 [27]
Ash content (total minerals) (%)	Dry ashing procedures using muffle a furnace set at 550°C for 6 hours	Miller-Ihli [28] Soderberg [24]
Lipid content (%)	Soxhlet extraction method using petroleum ether (Gravimetric method)	Soderberg [24] JORA n.33 [29]
Protein content (%)	Kjeldahl method for total kjeldahl nitrogen (TKN) determination	Soderberg [24] JORA n.37 [30]
Total sugar content (mg/L)	Colorimetric method	Dubois et al. [31] Soderberg [24]

TABLE 3
Bacteriological analysis of camel meat.

Bacterial parameters	Culture media	Bacterial isolation technique	Temperature and incubation time	References
TAMF	Plate Count Agar	Spread plate technique	30 and 22°C for 72 hours	JORA n.65 [32]
Coliforms	VRBL	Pour plate technique	30°C for 24 to 48 hours (Total coliforms) 44°C for 24 to 48 hours (Fecal coliforms)	JORA n.72 [33]
Coagulase-positive staphylococci (CoPS)	Baird-Parker agar	Spread plate technique	37°C for 24 hours	JORA n.68 [34] JORA n.14 [35]
Sulfite-reducing Clostridia (SRC)	Meat-liver agar	Anaerobic culture tube	46°C for 24 to 48 hours	JORA n.51 [36]
Fungal flora	-Rose-Bengal chloramphenicol agar (RBC) -Sabouraud 4% chloramphenicol dextrose agar	Spread plate technique	25°C for 3 to 5 days	JORA n.48 [37] JORA n.52 [38]
Salmonella spp	-Selective enrichment media: RVB, and SCB. -Hektoen agar, and SS agar	Enrichment on selective broth medium, and isolation on selective agar medium (streak plate technique)	41,5 and 37°C for 24 hours (Enrichment step). 37°C for 24 hours (Isolation on agar)	JORA n.44 [39]
Listeria monocytogenes	-Selective broth media: Half Fraser broth, and Fraser broth -Selective agar media: PALCAM Listeria agar	Enrichment on selective broth medium, and isolation on selective agar medium (streak plate technique)	37°C for 24 hours	Benyagoub et al. [10]
Bacillus spp	Mossel (MYP)	Pour plate technique	37°C for 24 to 72 hours	Rachedi et al. [40]
Pseudomonas spp	Cetrimide-Nalidixic acid agar	Spread plate technique	36 ± 2°C for 24 hours	Benyagoub et al. [10]

VRBL: Violet Red Bile Lactose Agar; MYP: Mannitol Egg Yolk Polymyxin Agar; RVB: Rappaport-Vassiliadis broth; SCB: Selenite Cystine Broth; SRC: Sulfite-reducing Clostridia; RBC: Rose-Bengal chloramphenicol; TAMF: Total Aerobic Mesophilic Flora; SS agar: *Salmonella-Shigella* agar.

In this study, across the 23 antibiotics tested, we mainly focused on the five (5) antibiotics recommended by the Ceva Santé Animale Association as drugs for use in antibiotic therapy of animals : Penicillin (P 10µg), Amoxicillin (AMX 10µg), Oxytetracycline (TE 30µg), Erythromycin (E 15µg), and Co-trimoxazole (SXT 25µg) [10].

According to the CLSI [46] guidelines, inhibitory zone diameters in (mm) produced around the antibiotic discs after incubation at 37°C for 18 to 24 hours were interpreted as sensitive, intermediate, or resistant.

Data analysis. The results of different experiments were expressed as the average value of two replicates. For microbiological analysis results, all colonies were counted and expressed in (n Log_{10} CFU/g and n CFU/g) depending on the microbial

parameter analyzed. The data were prepared using Excel 2016 software, in order to be more ready for processing in R statistical software (R statistics package 2023 version 4.3.1). Descriptive variables and differences in physicochemical and microbiological results between the test and control samples (two groups) were carried out using Student's t-test. While the level of significance was set at ($p<0,05$).

RESULTS

Physicochemical and microbiological analysis. The physicochemical and microbiological analysis results are shown in Tables 4, 5, and Figure 3.

TABLE 4
Physicochemical analysis results of dried and salted camel meat samples.

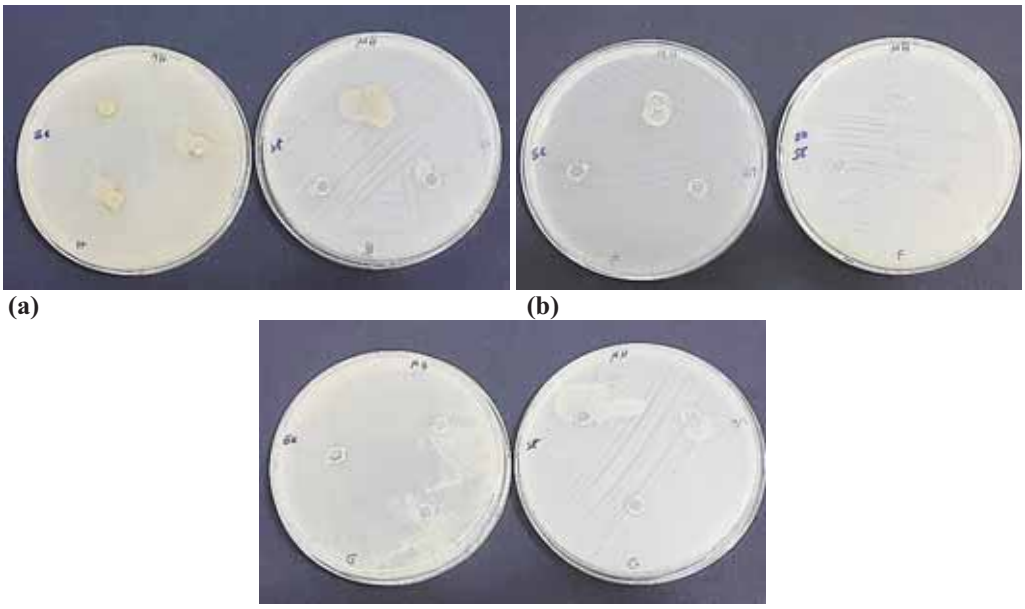
Samples	pH	TA (g/L)	Sodium chloride content (g/L)	WHC (%)	Moisture content (%)	TSC (%)	Ash content (% DW)	Lipid content (%)	Protein content (%)	Total sugar content (mg/L)	Detection of anti-biotic residues
A	5,43	3,4	1,3	199	22,8	77,2	13,7	23	24,24	59,16	**Qualitative test :** All fresh/ dried and salted camel meat samples analyzed are free of the following antibiotics (TE, AMX, P, E, and SXT)
B	5,36	3,45	3,31	267,05	6,46	93,54	38,58	22,68	36,17	73,04	
C	5,14	3,63	0,09	208	14,05	85,95	3,68	76,43	3,06	28,24	
D	5,59	3,2	0,65	248	10,64	89,36	9,54	19,11	31,4	21,3	
E	5,24	3,5	0,97	232	11,32	88,68	9,98	23,31	28	28,95	
F	5,26	3,56	0,68	226,83	10,73	89,27	9,56	15,1	28,87	32,66	
G	5,34	3,47	1,35	214,08	13,52	86,48	15,52	4,97	26,83	76,51	
H	5,4	3,43	0,49	265	8,9	91,1	7,12	12,08	32,52	50,6	
Min value	**5,14**	**3,2**	**0,09**	**199**	**6,46**	**77,2**	**3,68**	**4,97**	**3,06**	**21,3**	
Max value	**5,59**	**3,56**	**3,31**	**267,05**	**22,8**	**93,54**	**38,58**	**76,43**	**36,17**	**76,51**	
Mean value	**5,34**	**3,45**	**1,1**	**232,5**	**12,3**	**87,7**	**13,46**	**24,58**	**26,39**	**46,31**	
TS	5,45	3,38	0,15	162,52	25,34	74,66	5,78	7,63	21,73	20	
TF	5,72	3	0,12	99,33	76,75	23,25	4,73	3,51	18	16,68	
Sig. level	$0,301^{NS}$	$0,386^{NS}$	**0,026***	$0,169^{NS}$	$0,372^{NS}$	$0,372^{NS}$	**0,069***	**0,045***	$0,147^{NS}$	**0,0078***	NS

A to H: Dried and salted camel meat (test samples); TS and TF: Control samples (TS: Fresh camel meat/ TF: Dried camel meat); TA: Titratable acidity; TSC: Total solid content; WHC: Water-holding capacity; TE: Tetracycline; AMX: Amoxicillin; P: Penicillin; E: Erythromycin; SXT: Co-trimoxazole; DW: Dry weight; Sig. level: Significant level; NS: No significant; * = Significant at $p<0,05$; Min value: Minimum value; Max value: Maximum value; Source: Own study.

TABLE 5
Microbiological analysis results of dried and salted camel meat samples.

Samples	TAMF (n Log_{10} CFU/g)		Coliforms (n Log_{10} CFU/g)		Staph (n Log_{10} CFU/g)	SRC (spores/ 20mL)	FF (n Log_{10} CFU/g)	Sal (per 25g)	LM (per 25g)	*Bacillus* spp (n Log_{10} CFU/g)	*Pseudomonas* spp (CFU/g)
	22°C	30°C	TC	FC							
A	4,32	4,27	<1	<1	<1	<1	2,48	-ve	-ve	<1	-ve
B	4,23	4,02	2,48	<1	2,11	<1	3,45	-ve	-ve	<1	-ve
C	6,4	6,46	4,68	3,7	4,12	21	4,66	-ve	-ve	3,23	-ve
D	4,4	4,56	2,84	<1	3,53	<1	3,5	-ve	-ve	<1	-ve
E	5,18	6,48	3,19	<1	2,44	17	4,12	-ve	-ve	<1	-ve
F	4,49	4,44	3,15	1,48	4,08	12	3,59	-ve	-ve	<1	-ve
G	5,07	5,16	1,5	<1	4,42	09	4,17	-ve	-ve	<1	-ve
H	4,75	4,72	3,08	2,15	4,27	12	3,81	-ve	-ve	<1	-ve
Min value	**4,23**	**4,02**	**<1**	**<1**	**<1**	**<1**	**2,48**	/	/	**<1**	/
Max value	**6,4**	**6,48**	**4,68**	**3,7**	**4,42**	**21**	**4,66**	/	/	**3,23**	/
Mean value	**4,85**	**5,01**	**2,61**	**0,92**	**3,12**	**8,87**	**3,72**	/	/	**0,40**	/
JORA n.39 [43]	**5,7**		/	**2,7-3,7**	**CoPS (2,7-3,7)**	/	/	**-ve**	/	/	/
TS	4,48	4,55	5,21	<1	4,38	2	2,95	-ve	-ve	<1	-ve
TF	4,33	4,37	3,25	<1	6,57	<1	2,48	-ve	-ve	<1	-ve
Sig. level	$0,126^{NS}$	$0,153^{NS}$	$0,311^{NS}$	$0,107^{NS}$	**0,230***	**0,033***	**0,046***	NS	NS	$0,350^{NS}$	NS

A to H: Dried and salted camel meat (test samples); TS and TF: Control samples (TS: Fresh camel meat/ TF: Dried camel meat); FF: Fungal flora; Sal: *Salmonella* spp; LM: *Listeria monocytogenes*; Staph: Staphylococci; SRC: Sulfite-reducing Clostridia; *L. monocytogenes*: *Listeria monocytogenes*; TAMF: Total Aerobic Mesophilic Flora; CoPS: Coagulase-Positive Staphylococci; JORA: Official Journal of People's Democratic Republic of Algeria; -ve: Negative culture of presumed isolate on selective medium; Sig. level: Significant level; NS: No significant; * = Significant at $p<0,05$; Min value: Minimum value; Max value: Maximum value; For SRC, 20ml of stock solution is equivalent to 2g of dried and salted camel meat sample; <1: No bacterial growth; / : No specific bacterial load; Source: Own study.



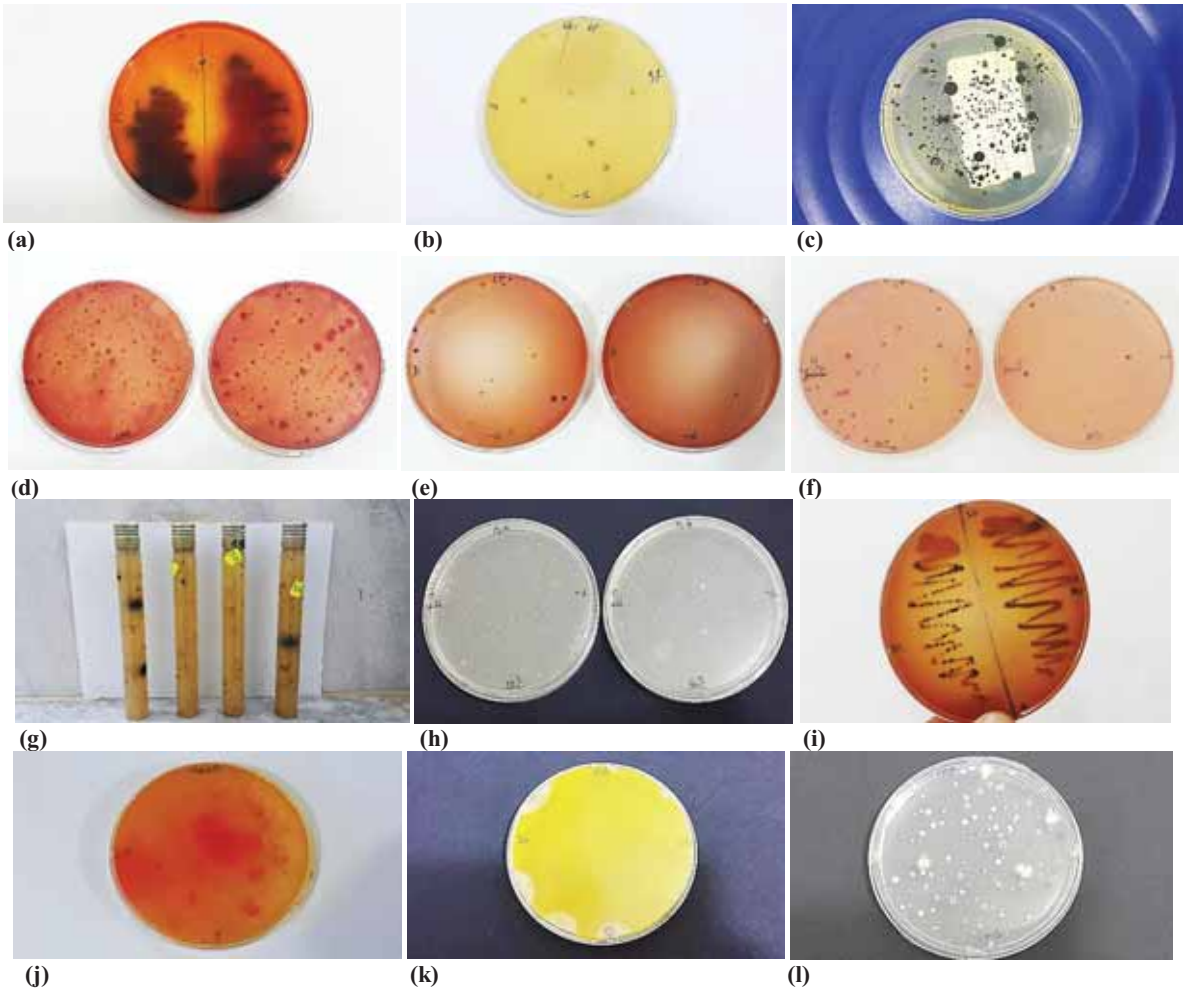
(a) **(b)**

(c)

FIGURE 4

Agar-well diffusion method on Mueller-Hinton agar medium (Original, 2023).

S.A.: *Staphylococcus aureus*; E.C.: *Escherichia coli*; (a): Sample H; (b): Sample F; (c): Sample G.



(a) **(b)** **(c)**

(d) **(e)** **(f)**

(g) **(h)** **(i)**

(j) **(k)** **(l)**

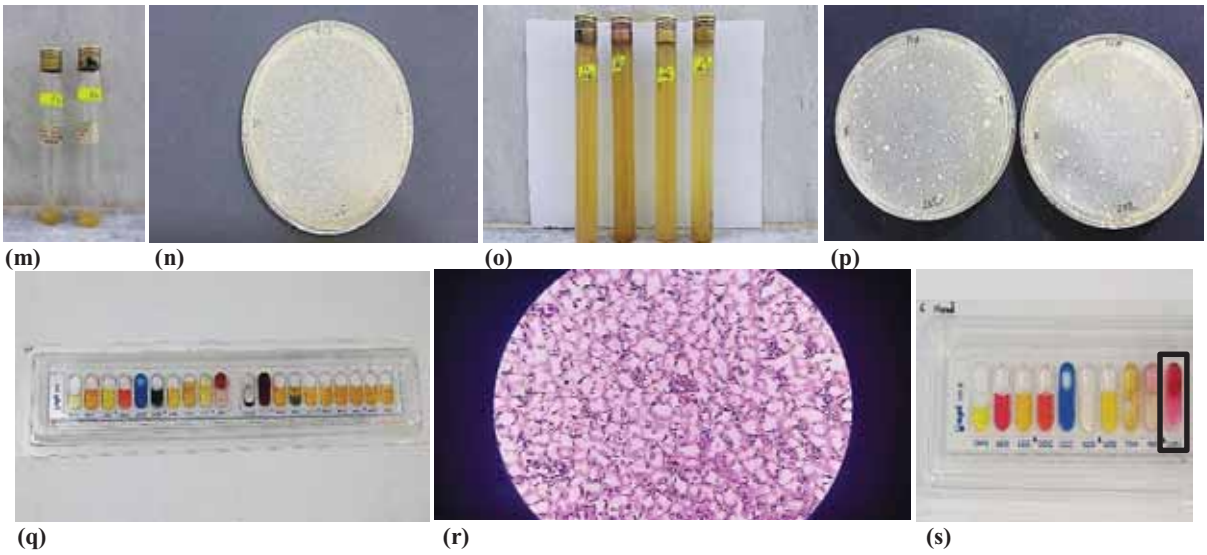


FIGURE 3

Illustration of the bacteriological analysis of dried and salted camel meat (Original, 2023).

(a): Suspected presence of *Listeria* bacteria on Palcam agar; (b): Staphylococci strains on Baird-Parker agar (Sample B); (c): Staphylococci strains on Baird-Parker agar (Sample D); (d): Fecal coliforms on MacConkey agar (Sample B); (e): Total and fecal coliforms on MacConkey agar (Sample D); (f): Total coliforms on MacConkey agar (Sample TF); (g): Sulfite-reducing Clostridia (Sample G); (h): TAMF on PCA medium (Sample TS); (i): Suspected presence of *Salmonella* sp on SS agar; (j): Suspected presence of *Bacillus* spp on Mossel agar (Sample F); (k): Suspected presence of *Bacillus* spp on Mossel agar (Sample C); (l): Fungal flora of PDA medium; (m): Tube coagulase test; (n): TAMF on PCA medium (Sample C); (o): Sulfite-reducing Clostridia (Sample TF); (p): TAMF on PCA medium (Sample E); (q): API 20E multitest (Sample TF); (r): Microscopic appearance of *Bacillus* spp (Gram stain); (s): Voges-Proskauer and other tests for staphylococcal isolates.

TABLE 6

Identification of pathogenic bacterial species isolated from dried and salted camel meat samples.

Samples	*Salmonella* spp	*L. monocytogenes*	*Bacillus* spp	Coagulase-positive staphylococci (CoPS)
A	-ve (*Kluyvera* spp)	-ve	-ve	-ve (CoPS) -ve (CoNS)
B	-ve (*Enterobacter cloacae)*	-ve	-ve	
C	-ve (*Serratia odorifera* ; *Escherichia coli*)	-ve	+ve (*Bacillus cereus*) Mann (-); Lecith (+)	-ve CoPS
D	-ve (*Enterobacter sakazakii*)	-ve	-ve	+v CoNS, VP+ (*S. saprophyticus, S. epidermidis*)
E	-ve (*Enterobacter sakazakii*)	-ve	-ve	
F	-ve (*Citrobacter freundii* ; *Escherichia coli*)	-ve	-ve	
G	-ve (*Enterobacter cloacae)*	-ve	-ve	
H	-ve (*Serratia fonticola* ; *Escherichia coli)*	-ve	-ve	
TS	-ve (*Enterobacter aerogenes*)	-ve	-ve	
TF	-ve (*Enterobacter sakazakii*)	-ve	-ve	

CoPS: Coagulase-Positive Staphylococci; CoNS: Coagulase-Negative Staphylococci; -ve: Negative culture of presumed isolate on selective medium; +ve: Positive culture of presumed isolate on selective medium; *S. saprophyticus*: *Staphylococcus saprophyticus*; *S. epidermidis*: *Staphylococcus epidermidis*; VP+: Positive Voges-Proskauer test; Mann (-) : Negative mannitol fermentation; Lecith (+): Positive lecithinase test (Nagler's Reaction); Source: Own study.

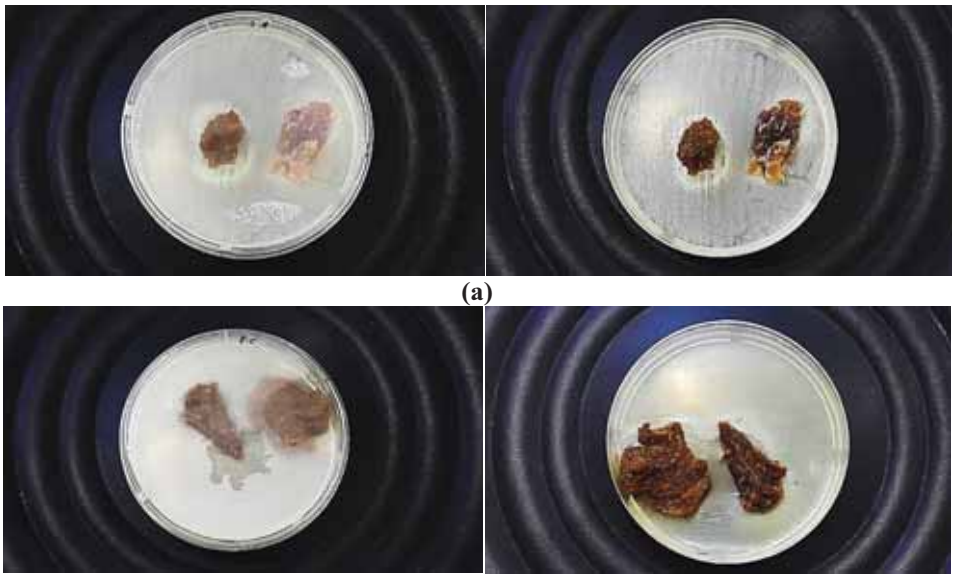
Bacterial identification. Various biochemical identification tests made it possible to confirm the absence of presumed pathogenic bacteria, while the identification results of the isolated bacteria are shown in Table 6.

Detection of antibiotic residues. The qualitative test for the detection of antibiotic residues carried out using the agar-well diffusion method showed no inhibitory effect on the growth of *E. coli* and *S. aureus* for all samples analyzed compared to

the antibiotic susceptibility testing using antibiotic disks (Table 7, and Figures 4, 5 and 6).

In addition, the antibiotic test kit showed no trace of antibiotics in the stock solution of dried and salted camel meat samples (Figure 7). This can be explained by the absence of inhibitory agents including antibiotic residues (Tetracyclines, beta-lactams, includind cephalexin).

Antibiotic susceptibility testing results for reference bacterial strains showed that *E. coli* was resistant to co-trimoxazole, imipenem, ampicillin, amoxicillin, cefoxitin, tetracycline, and amoxicillin-clavulanic acid, while *S. aureus* was resistant to penicillin, oxacillin, cefoxitin, erythromycin, and tetracycline.



(a)

(b)

FIGURE 5

Diffusion method on Mueller-Hinton agar medium (Original, 2023).

(a): *Staphylococcus aureus* ATCC 25923.

(b): *Escherichia coli* ATCC 25922.

(a) *Escherichia coli* ATCC 25922 **(b)** *Staphylococcus aureus* ATCC 25923

FIGURE 6

Antibiotic susceptibility testing using the disc diffusion method on Mueller-Hinton agar medium (Original, 2023).

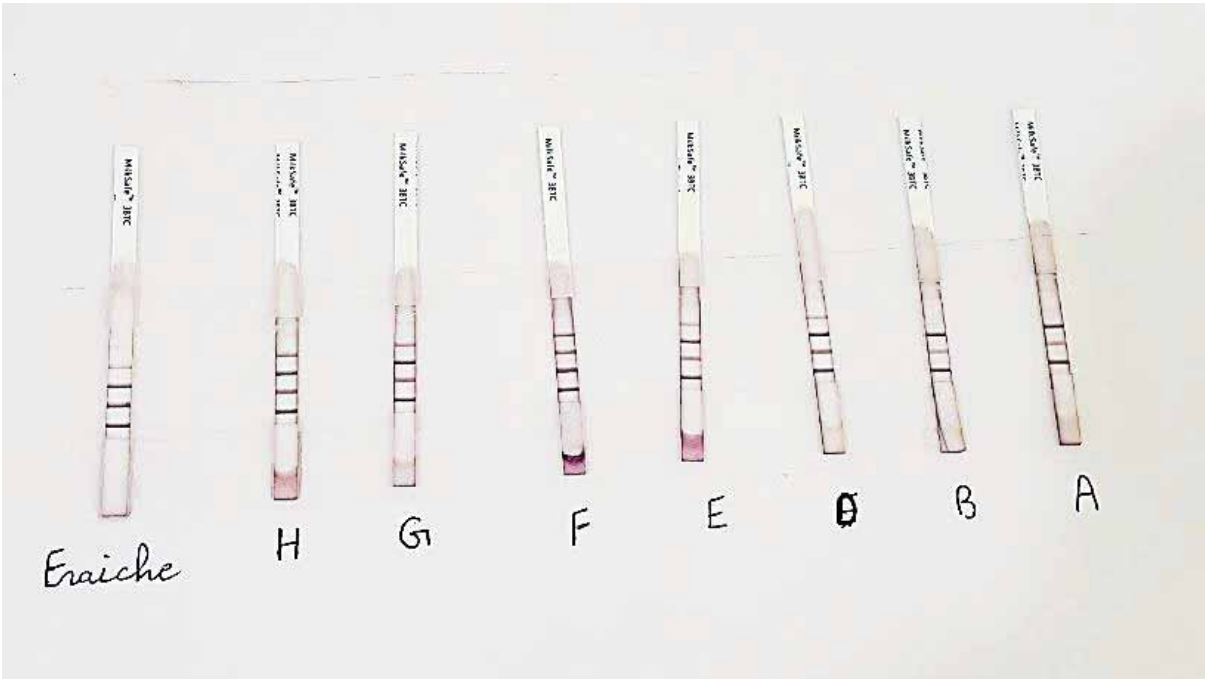


FIGURE 7
Antibiotic test kit results (Original, 2023).

TABLE 7
Multidrug-resistant profiles of the tested reference bacterial strains.

Reference bacterial strains	Antibiotics
***E. coli* ATCC 25922**	**TE-FOX-AMC-IPM-SXT-AMP-AMX** & Susceptible to FOS-C-AK-CN-NET-CAZ
***S. aureus* ATCC 25923**	**E-TE-P-FOX-OX** & Susceptible to SXT-C-OFX-RA-CIP-CN-LEV-VA-CD

E. coli: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*; ATCC: American Type Culture Collection; AMC 30µg: Amoxicillin-clavulanic acid; P 10µg: Penicillin; AMX 25µg: Amoxicillin; TE 30µg: Tetracycline; E 15µg: Erythromycin; SXT 25µg: Co-trimoxazole; CN 10µg: Gentamicin; FOS 50µg: Fosfomycin; CIP 5µg: Ciprofloxacin; RA 5µg: Rifampin; OFX 5µg: Ofloxacin; OX 5µg: Oxacillin; C 30µg: Chloramphenicol; LEV 5µg: Levofloxacin; IPM 10µg: Imipenem; AMP 10µg: Ampicillin; AK 30µg: Amikacin; FOX 30µg: Cefoxitin; VA 30µg: Vancomycin; NET 30µg: Netilmicine; CAZ 30µg: Ceftazidime; CD 2µg: Clindamycin; VA 30µg: Vancomycin; Source: Own study.

DISCUSSION

Physicochemical and biochemical analyses. The pH value is an important indicator for assessing meat quality which has an impact on some factors such as meat tenderness and shelf life of the product [3].

The dried and salted camel meat samples analyzed showed pH values ranging from 5,14 to 5,59, and total titratable acidity ranging from 3,2g/L to 3,63g/L against pH of 5,72 and 5,45, and total titratable acidity values of 3 and 3,38g/L for TF and TS as control samples, respectively.

Benyoucef and Bouzegag [8]; Kadim et al. [12]; Benaissa [17]; Ould El hadj et al. [47]; Smili et al. [48], and Babiker and Yousif [49] reported that the pH of fresh camel meat decreases over time and moves from a pH of 6 (4 hours after slaughter) to pH values ranging from of 5,47 to 5,73 after 24 hours, respectively. These values were found to be less acidic compared to the pH of other animal species as reported by Hamani et al [50] for beef (5,58–5,74), resulting from differences in muscle fiber types, lower muscle glycogen in the meat, decreased level of muscle glycolytic enzymes (gluconeogenesis), metabolism of the muscles, linked to the age of the animal, the breed and muscle type [3, 8, 48, 51-53]. Benlacheheb et al. [54] reported that pH can also decrease during the drying period, and then remain almost unchanged during the rest of the ripening period.

The results obtained for sodium chloride content showed values ranging from 0,09g/L to 3,31g/L, with an average value of 1,105g/L. The chloride sodium content of dried and salted camel meat samples recorded significant differences ($p<0,05$) between the test and control samples (Table 4). Benlacheheb et al. [54] reported that dried and salted meat, known as El Gueddid, showed high salt content. In this study, except for sample (C), the recorded values for sodium chloride exceed those given by Amimour and Santouh [55] which were 0,11mg/L and 0,5mg/L. This difference is mainly due to the use of different amounts of salt during the salting operation of the samples. These results are in line with the report of Benyagoub and Bessadet [19] that the concentration of minerals in processed meat increased with the addition of salts and seasonings.

It should be noted that salting extends the shelf life of foods. By absorbing a large amount of free water, salt increases osmotic pressure, reduces water activity (Aw), and therefore makes the survival of microorganisms difficult [16, 56, 57].

Water absorption capacity is the ability to hold water against gravity and includes bound water, hydrodynamic water, capillary water, and physically trapped water [58]. The amount of water associated with proteins is closely related to their amino acid profile and increases with the number of charged residues [59], conformation, pH, temperature, ionic strength, and protein concentration [60, 61]. According to Smili et al. [48], water-holding capacity and pH seem highly interdependent, as phenomena associated with muscle acidification, post-mortem proteolysis, and sarcolemma alteration contribute to water release.

Amimour and Santouh [55] reported a WHC rate of 450% for the dried camel meat analyzed. This is a very high rate compared to the results obtained, which range from 199% to 267,05% with an average value of 232,49%. These results suggest that the composition and structure of dried camel meat samples, including carbohydrate content and protein distribution, may influence their ability to absorb water. This can have effects on the texture and quality of the meat by-product (Kadid). The amount of moisture content in the meat, variations in fat content, the binding ability of meat proteins, and the pH of the muscle influence the water-holding capacity and water activity of the meat [12, 62].

Although it does not provide any energy value to the food, water plays a very important role that affects the structure, appearance, and taste of foods, and their susceptibility to degradation, so its determination is therefore very important. The shelf life of a product depends on the amount of water it contains [63].

The water content of the analyzed samples varied from 6,46% to 22,8% with an average value of 12,3%, where sample (A) and control sample (TS) recorded significant water content (22,8% and 25,34%), respectively. According to Benlacheheb et al. [54], the physicochemical characteristics of El Gueddid showed that the moisture content decreased in the product during the drying period (low water activity). Amimour and Santouh [55] reported low moisture content in dried and salted camel meat (0,48% – 0,64%). The data provided by Muhammad et al. [9] on similar products prepared with camel meat such as *Kilishi* and *soye* showed moisture content of 10,3% and 36,91%, respectively.

The decrease in moisture content is due not only to the drying of the meat but also to the effect of salt added during the preparation of the dried meat [19]. The resulting moisture loss tends to concentrate the nutrients in the final products [9]. The moisture content plays a vital role in the processing performance, shelf life, and quality characteristics of camel meat [3].

The results of our previous studies [10] and those obtained by Abd El-Hady et al. [2]; Muhammad et al. [9]; Ould El hadj et al. [47]; Babiker and Yousif [49], and Fakolade et al. [62] showed that the average moisture content in fresh camel meat was 79%, 73,89%, 76,77%, 75,98%, 75,64%, and 76,01%, respectively, which is similar to the result of this study. This amount of water makes this meat vulnerable to several microbial alterations [47] and thus requires preservation.

The low moisture content in Kadid is due, firstly, to the drying process that removes a large amount of water, and secondly to salting, which plays an important role in reducing water activity in foods (Salting out effect) [19].

According to Benyoucef and Bouzegag [8], the dry matter content of fresh camel meat increases with the age of the animal (23% to 25,2%). This supports the result we obtained (23,25%), and after drying, the dried and salted camel meat samples experienced an increase in dry matter by removing water (77,2% to 93,54%).

The ash content results in the samples analyzed were variable from one sample to another, a high content is recorded for sample B (38,58%), while the other samples showed that the ash content ranged from 3,68% to 15,52%. According to Nganguem [64], the total ash content of food makes it possible to estimate the level of minerals it contains. The results obtained exceed the data on ash content reported by Muhammad et al. [9] for similar products prepared with camel meat, namely *Kilishi* and *soye* (4,7% and 3,4%), respectively. The results reported by Ould El hadj et al. [47]; Fakolade et al. [62]; Babiker and Yousif [49]; Kamoun [65]; Bouras and Moussaoui [66], and Abd El-Hady et al. [2] showed that fresh camel meat contains an ash content ranging from 1,01% to 1,21%. While values ranging from 0,95% to 6,7% were reported for dried beef by Hamani et al. [50].

The high ash content in Kadid can be attributed to the salts and ingredients added during the preparation of this product [19, 67].

It should be noted that the ash content in foods can vary depending on several factors, such as production methods, and ingredients used. The amount of ash in a food can also be affected by the presence of minerals found naturally in animal tissues.

Based on dry weight (DW), the dried camel meat samples analyzed had a fat content ranging from 4,9% to 23,3% which is lower than the results reported on similar products prepared with camel meat, namely *Kilishi* and *soye* (24,7% and 29,1%), respectively [9]. While on a fresh weight (FW) basis, Kadim et al. [12]; Benaissa [17], and Babiker and Yousif [49] reported that fresh camel meat is relatively lean, containing only 0,92% to 1,01% fat.

The results given by Ould El hadj et al. [47], and Babiker and Yousif (1990) [49] showed that camel meat contains an average fat content of 1,49% and 1,41%, while Abd El-Hady et al. [2], and Kamoun [65] reported finding a value of 2,63% and 2,6%, respectively. The fat content of dried and salted camel meat samples recorded significant differences ($p<0,05$) between the test and control samples (Table 4).

These differences in fat content can be due to several factors, such as the difference in the method of sampling in the first place (i.e. depending on the part from which the sample was taken), feeding conditions of the animals, and husbandry practices. It should also be noted that fat content can vary depending on different parts of the meat (e.g. lean versus fatty cuts), the age of the animal, the breed, and the processing and preparation techniques used. For this, it is recommended to take these variations into account when evaluating the fat composition of camel meat [12, 17].

Samples of dried and salted camel meat showed a protein content ranging from 21,7% to 36%. These results are rather high compared to the results reported by Abd El-Hady et al. [2], Muhammad et al. [9], Kadim et al. [12], Babiker and Yousif [49], Fakolade et al. [62], Kamoun [65], and Bouzgag [68], which were between 16% and 22% (FW) protein on a fresh weight basis. It should be noted that the protein content in camel meat can vary depending on several factors, such as the age of the animal, diet, breeding conditions, and processing methods [8, 10, 19].

The total sugars content in dried and salted camel meat ranges from 0,385% to 1,53% (20mg/L – 76,51mg/L). Ould El hadj et al. [47] reported a total sugar content of 1,2%. The sugar total content of dried and salted camel meat samples recorded significant differences ($p<0,05$) between the test and control samples (Table 4).

The presence of total sugars in meat can be attributed to several factors. First, sugars may be naturally present in camel meat, either due to the animal's metabolism or diet. Some sugars can be present in muscle tissues as a source of energy for the animal [47]. Furthermore, sugars may be added during the Kadid preparation process, marinating, or seasoning stage. Sauces or ingredients used to flavor meat may contain added sugars, which may increase the total sugar content. Factors such as preparation method, shelf life, storage conditions, temperature, moisture content, and the presence of enzymes can affect the breakdown of sugars and therefore their concentration in meat [69, 70].

According to Si et al. [3], and Suliman et al. [52], the chemical composition and quality characteristics of camel meat vary according to age, sex, muscle type, breed type, feeding conditions, and site on the carcass.

The results of the detection of antibiotic residues through various qualitative tests showed that all samples analyzed were free of antibiotic residues. This is probably due to compliance with strict withdrawal periods (21 days) recommended by veterinarians after antibiotic treatment to ensure that milk, and meat are kept out of the food supply (banning camel slaughter and milking), and is also consistent with WHO guidelines on the use of antimicrobials in food-producing animals [71]. This reinforces the strong trend of camel meat as one of the safe foods with multiple health claims [5, 72, 73].

Antibiotic susceptibility testing of reference bacterial strains on MH agar was used as a comparison tool to determine the nature of antibiotic residues present in the sample. Despite the negative qualitative results for antibiotic residues, the results of antibiotic susceptibility testing were similar to previous studies [74, 75].

Microbiological analysis. Dried and salted camel meat samples were subjected to microbiological analysis against two control samples according to national regulations.

The microbiological analysis results indicate that the total flora for three samples (C, E, and G) exceeds the load recommended by the national and international standard set at 5 Log_{10} CFU/g. The aerobic plate count is a measure of the microbial quality of the meat. Any exceeding of this load could be a spoilage indicator [10, 76].

From the results obtained for TAMF and fungal flora loads, the samples showed a TAMF loading ranging from 4,02 to 6,48 Log_{10} CFU/g where samples C, F, G, and H revealed a higher loading of total aerobic bacteria compared to that reported by Hamad [77] who recorded a microbial load of 1,79 Log_{10} CFU/g of camel carcass at El Oued slaughterhouse. The results of the fungal flora of dried and salted camel meat samples recorded significant differences ($p<0,05$) between the test and control samples (Table 5).

This microbial load can arise for several reasons, namely : slaughtering conditions and the method of skinning camels, the conditions for transporting meat samples and preparing dried meat [55].

The results of the search and enumeration of coliform counts revealed a fecal coliform load ranging from 1,48 to 3,7 Log_{10} CFU/g, where 7 samples including the control samples were fecal coliforms-free, while one sample (C) exceeded the threshold set by national regulations. This is a significant contaminant load compared to data reported by Hamad [77], who recorded an overall total coliform contamination load of 0,84 Log_{10} CFU/g in camel carcasses.

Contamination of carcasses appears to be inevitable during slaughter operations, especially during evisceration, by workers' hands, equipment,

water used, or by possible rupture of the gastric reservoir [78].

According to our results, 7/10 (i.e. 70%) of the samples were free of fecal coliform 'EC'. However, samples C, F, and H were contaminated with an *E. coli* load of 3,7; 1,48, and 2,15 Log_{10} CFU/g, respectively. A load that falls within the limit set by national regulations [43] requires an acceptable limit level (m) i.e. 2,69 Log_{10} CFU/g and a risk contamination level (M) not exceeding 3,7 Log_{10} CFU/g. Hamad [77], revealed a contamination level of 0,5 Log_{10} CFU/g for camel carcass.

Fecal coliform contamination indicates poor hygienic conditions and refers in particular to fecal contamination and thus defects occurring during evisceration or unsanitary behavior of handlers during the preparation of Kadid [19, 79].

Sulfite-reducing anaerobes are generally Clostridia, whose spores are found in outdoor environments and during the slaughter of animals. They are considered as test bacteria to evaluate the health quality of foodstuffs of animal origin. They are sometimes used as an indicator of fecal contamination, which is the cause of food infections [80], but their presence does not always indicate this origin [81].

According to the results obtained, 6/10 (i.e. 60%) of the samples were contaminated with sulfite-reducing Clostridia spores loads ranging from 2 to 21 spores/20mL (the volume of sample analyzed is equivalent to 2g of dried and salted camel meat), with no *Bacillus* spp except for sample C which had a *B. cereus* load of 3,23 Log_{10} CFU/g most likely due to the low quality of *Dhane* (traditional butter) added to the ground camel meat according to the preparation recipe followed by families in the province of Tindouf (Far southwestern Algeria) [19]. For statistical analysis, dried and salted camel meat samples recorded significant differences ($p<0,05$) for the SRC parameter between the test and control samples (Table 5). Amimour and Santouh [55]; Hamad [77], and Boudadi et al. [82] reported the absence of sulfite-reducing Clostridia spores. Since meat drying is often done outdoors, this could justify contamination with sulfite-reducing Clostridia spores which are indicators of telluric contamination [83, 84, 85].

The fecal contamination detected was probably a result of a lack of hygiene in handling, and storage conditions [10, 86].

Staphylococci present a real risk to the consumer when the load in the product is very high, but also poses a potential risk when the contaminated product is stored in conditions that allow their spread [78]. Their presence may indicate poor storage conditions in terms of handling and hygiene of handlers because they are bacteria frequently found on the skin and mucous membranes of humans [87, 88].

The isolation and identification results of staphylococci revealed contamination of the analyzed samples with *S. saprophyticus*, and *S. epidermidis* as coagulase-negative staphylococci (CoNS) with loads ranging from 2,11 to 4,42 Log_{10} CFU/g, compared to a load of 6,57 Log_{10} CFU/g for the TF control sample. This bacterial load supports the finding of Benyagoub et al. [10], and Benlacheheb et al. [54], where staphylococci and lactic acid bacteria were the most abundant micro-organisms in the product.

Staphylococci results of dried and salted camel meat samples recorded no significant differences ($p>0,05$) between the test and control samples (Table 5).

This depends not only on the initial contamination levels and the temperature conditions under which the carcasses are stored [89] but also on the preparation of the dried meat. These results support the data reported by Amimour and Santouh [55]; Hamad [77], and Boudadi et al. [82] who noted the absence of coagulase-positive staphylococci.

Loads of coliforms and CoNS showed a significant decrease or even the absence of these bacterial contaminants in some samples of dried and salted camel meat compared to fresh camel meat. This supported the findings of Benlacheheb et al. [54], where any coliforms already present at low loads will then be eliminated after the post-salting stage of the process.

The results of the search for pathogenic bacteria showed that all samples were free of *Salmonella* and *L. monocytogenes*, where the isolated bacterial strains were identified as follows : *Kluyvera* spp, *Enterobacter cloacae*, *Escherichia coli*, *Enterobacter sakazakii*, *Serratia fonticola*, *Serratia odorifera*, *Citrobacter freundii*, and *Enterobacter aerogenes*. This met the recommendation of national regulations [43] that require its absence in 25g of meat.

The results also showed the absence of *Pseudomonas* spp. These results are similar to previous data on fresh camel meat [10, 90] where the method of preparing this traditional product, the ingredients, the drying stage, and the availability of natural antagonists against pathogenic species could be factors contributing to the elimination of these pathogens [10, 19, 91, 92].

Except for the FF and SRC parameters, no significant differences ($p>0,05$) were recorded for all other microbial parameters (TAMF, coliforms, staphylococci, *Salmonella* spp, *L. monocytogenes*, *Bacillus* spp, and *Pseudomonas* spp) between the test and control samples (Table 5).

It should be noted that some of the bacterial parameters analyzed in this study do not have a specific bacterial load reported in the national regulation. However, this regulation [43] indicates that a sample is considered toxic if the bacterial load of the sulfite-reducing anaerobic bacteria, and

Bacillus cereus is equal to or greater than 5 Log_{10} CFU per gram or mL of sample tested.

Although some of the samples analyzed were contaminated with coliforms falling within the maximum limit set by national regulations, with no pathogenic bacteria, the product had good preservation ability and could contribute to food security and food safety in arid regions, because from the point of view of economic profitability, camel meat presents a competitive advantage compared to other meats due to their low production cost [73].

Limitation and suggestions. This study was limited by analyzing some samples of dried and salted camel meat prepared in three provinces located in southwestern Algeria. We suggest analyzing other samples from the southern provinces of Algeria, where different camel breeds are available and their meat could affect the quality of dried and salted camel meat. Furthermore, the detection of antibiotic residues was limited by qualitative testing. These data need to be further confirmed using HPLC analysis.

CONCLUSION

The analyzed dried and salted camel meat samples showed interesting physicochemical and biochemical characteristics, as the method of preparing this type of meat product not only contributed to the good preservation of the product (storage at room temperature) but also ensured the promotion of its nutritional and sensory quality. The absence of pathogenic bacteria namely : *S. aureus* (CoPS), *Pseudomonas* spp, *L. monocytogenes*, and *Salmonella* spp, as well as the negative result for antibiotic residues, indicates that the hygienic quality of the analyzed samples complies with national regulations.

Improving the quality of this product depends not only on the stages of its preparation but also on the quality of the raw material, for which pre- and post-mortem factors must be carefully monitored from the slaughterhouse to the butchers.

This product and many others form part of a rich cultural heritage of southern Algeria, which must be preserved and enhanced on an industrial scale, to become one of the pillars of food security in arid regions.

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THE BENEFIT EFFECT OF SALICYLIC ACID ON PHYSIO-BIOCHEMICAL CHARACTERS OF FABA BEAN (*Viciafaba* L.) UNDER LEAD STRESS

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ABSTRACT

Lead (Pb) is a widespread toxic heavy metal that usually causes deleterious effects on plant growth and development.Salicylic acid (SA) is a natural signaling molecule, which participates in the regulation of physiological processes in plants such as growth, photosynthesis and other metabolic processes .A protective role of salicylic acid is well known under abiotic stress conditions. Therefore, the objective of this study was undertaken to evaluate the effect of different concentrations of salicylic acid(0.5, 1mM) a promising plant development regulatory substance in alleviating the deteriorative effect of lead at 50 µM on faba bean(*Vicia faba* L.).The results revealed that Pb stressed faba bean plants. In oter words, Pb supplementation decreased the growth characteristics (plant height, root length, root and shoot fresh weights)and photosynthetic pigments (chlorophyll a,chlorophyll b, carotenoids). Meanwhile, it increased malondialdehyde(MDA), Hydrogen peroxide (H_2O_2),osmotic solutes(proline and total soluble sugars) and antioxidant enzymes activities including ascorbate peroxidase and catalase. However, the data provided evidence that SA reduced the deleterious effect of Pb stress on faba bean plants. So, it might play a key role in providing stress tolerance by stimulation of the enzymatic antioxidant system as a stress protection mechanism.

KEYWORDS:
Viciafaba .L, lead, salicylic acid, antioxidants, osmotic, malondialdehyde

INTRODUCTION

Like all living beings, plants are immobile in their nature, and they are frequently expoed to various abiotic and biotic stresses, of which heavy metals stress represents one of the most damagining abiotic stress.Lead (Pb), a heavy metal with characteristic toxic actions, has attracted considerable attention for its widespread distribution and potential risk to the environment [1]. it gets easily absorbed and accumulated in various parts of the plant.The high accumulation of Pb in plant causes negative effects on nutrient uptake, photosynthesis, redoxbalance,antioxidant enzymes, respiration, membrane permeability, and protein synthesis [2, 3]. In addition, Pb toxicity in plants can cause phenotypic changes, including reduction in the root length, blackening of roots, plant growth retardation, yellowing of leaves, and disturbance of water balance [4].

Based these negative effects, plants have developed different adaptive mechanisms, and enzymatic antioxidants system plays important role in this challenge. For example, ascorbate peroxidase (APX) and catalase (CAT) help regulate cellular redox homeostasis at safe levels

[5,6,7].However, under Pb stress, excessive production reactive oxygen species (ROS) such as superoxide anion ($^{\bullet}O^{-}$), hydroxyl ion ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) disturbs this redox equilibrium [8], where ROS rapidly attack and oxidize biomolecules such as proteins, lipids, and nucleic acids, leading to plant cell death and growth retardation [9,10]. Clearly, plant response to Pb stress is a key research problem,and a viable and cost-effective method to eliminate Pb from the environment is needed .

Phytohormones have gained much attention over the last two decades due to their wide use in plants against many abiotic stresses[11]. Among them, salicylic acid (SA) is a very simple phenolic compound plays an important role in induction of plant defense against a variety of abiotic stresses through morphological, physiological and biochemical mechanisms. The positive effects of salicylic acid in mitigating stress are attributed to nutrient uptake improve, photosynthesis repair, membrane protection, it can interplays also with ROS signal pathways and reduces the effects oxidative stress[12,13].Many studies indicated that SA alleviates heavy metal toxicity such as Hg, Cd and Pb in plants [14,15,16,17,18].In addition, the signalling

roles of SA depend on many factors, including application mode, the exogenous and endogenous levels of SA and plant species [19].

The faba bean or broad bean, *Vicia faba*L., is considered one of the oldest leguminous crops in the world. Globally, it is the third most important feed grain legume [20].Its highly nutritional seeds are a crucial source of proteins, fiber, vitamins , minerals and carbohydrates for human consumption. According to the agricultural requirements, the main fields of interest in faba bean cultivation are yield improvement, disease resistance, abiotic stress tolerance and seed quality [21].

Thus, the present study was carried out to explore the potential role of SA in the mitigation of Pb toxicity.So, several parameters related to growth (plant height, root length, root and shoot fresh weights), photosynthetic pigments (chlorophyll a,chlorophyll b, carotenoids), oxidative stress (MDA andH_2O_2), antioxidant system (CAT and APX activities), osmotic solutes(Pro, TSC)were evaluated upon exposing faba bean plants to Pb stress with and without SA treatment.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The present study was carried out at the department of biology, Mohamed Cherif Messaadia university Souk-Ahras, Seeds of *Viciafaba* .L were germinated in the dark at 25°C for 7 days.After germination, plants were transferred to separated containers for hydroponics. Three-liter pots were used, containing ten plants each. Plants were grown in a controlled climate room at 24±2 °C and 50 % relative humidity, with a photoperiod of 16 h and a light intensity of 150µmol m^{-2} s^{-1}. The hydroponic medium consisted of Hoagland nutrient solution [22].The medium was continuously aerated (100 ml min^{-1}) and replaced every 3 days. At three-leave stage, the seedlings were treated by different concentrations of lead (Pb)as lead nitrate ($PbNO_3$) and salicylic acid (SA). The treatments were comprised of (i) 0 mM SA + 0 µM Pb (control), (ii) 0.5 mM SA + 0 µM Pb, (iii) 1 mM SA + 0 m Pb, (iv) 0 mM SA + 50µM Pb, (v) 0.5 mM SA + 50µ M Pb and (vi) 1 mM SA + 50µM Pb.The experiment was laid out in a completely randomized design with three replicates. Finally, the plant leaves were collected for analysis after 14 days of application of Pb and/or SA.

Analytical methods. Growth parameters determination. The plants were washed, fresh weight was determined using sensitive electrical balance and roots and shoots separated. Root and shoot lengths were measured by using meter scale.

Chlorophyll and carotenoid determination. Contents of assimilation pigments (chlorophyll a, chlorophyll b, carotenoids) were determined infresh weight of leaves by sample homogenization with 80% acetone. The absorbance was measured at 663 nm for chlorophyll a, 645 nm for chlorophyll b and 470 nm for carotenoids. Pigments contents were calculated using the equations of Lichtenthaler and Wellburn (1983) [23].

Osmotic solutes determination. The proline in leaves was estimated by the method of Bates *et al*. (1973). [24].The absorbance was measured spectrophotometerically at 520 nm and toluene was used as blank. The method of Bradford (1976) [25]. was employed for the estimation of protein in leaves. The absorbance was measured at 595 nm using bovine serum albumin as blank.The soluble sugars were determined by the method of Schields and Bunnet (1960) [26]. using anthrone in sulfuric acid.

Lipidperoxidationmeasurement. The level of lipid peroxidation was measured by estimating malondialdehyde (MDA, a product of lipid peroxidation) depending on the method of Heath and Packer (1968) [27]. Leaf samples (0.5 g) were homogenized in 3mL 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 10000×g for 10min. Then, the supernatant (1mL) was mixed with 4mL thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA), heated in a water bath at 95°C for 30min andquickly cooled by transferring in ice bath. After that, MDA content was measured by observing the difference in absorbance at 532nm using an extinction coefficient of 155mM^{-1} cm^{-1} and expressed as nmol of MDAg^{-1} FW.

Hydrogen peroxide content estimation. Hydrogen peroxide (H_2O_2) levels were determined according to Sergiev *et al*. (1997) method. [28]. Leaf tissue (0.5 g) was homogenized in an ice bath with 5 mL 0.1% (w/v) TCA. Subsequently the homogenate was centrifuged at 12,000 g for 15 min and 0.5 mL of the supernatant was added to 0.5 mL potassium phosphate buffer (10 mM, pH 7.0) and 1 mL potassium iodide (KI) (1 M). The absorbance of the supernatant was measured at 390 nm, the concentration of H_2O_2 was obtained using a standard curve and expressed as µmol g^{-1} FW.

Enzymes extraction and activities determination. Leaves (0.5g, fresh weight) were homogenized in 50 Mm potassium phosphate buffer pH 7.6. Then, the homogenized samples were centrifuged at 12000×g for 20 min and the supernatant crude extract was used for the estimation of both catalase (CAT) and ascorbate peroxidase (APX) activities [29]. CAT activity was determined as a decrease in absorbance at 240 nm for 3 min following decomposition of H_2O_2 [30]. The reaction mixture 3 ml contained 50mM phosphate buffer pH 7.2, 15 mM $H_2O_2$and 100 µl of crude enzyme extract. Enzyme

was quantified using the extinction coefficient 39400 $M^{-1}cm^{-1}$. Ascorbate peroxidase (APX) activity was determined following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 3 min in 3 ml of a reaction mixture containing 50 Mm potassium phosphate buffer pH 7.2, 0.5 Mm ascorbic acid, H_2O_2 and 100 µl of crude enzyme extract [31]. Enzyme was quantified using the extinction coefficient $2800M^{-1}cm^{-1}$.

Statistical analysis. The results presented are the means ± standard error of threereplicates (n = 3). The results were statistically confirmed by analysis of variance (ANOVA). Tukey's HSD test was applied to find means are significantly different from each other at $p \leq 0.05$ level using Minitab 16Statistical Software.

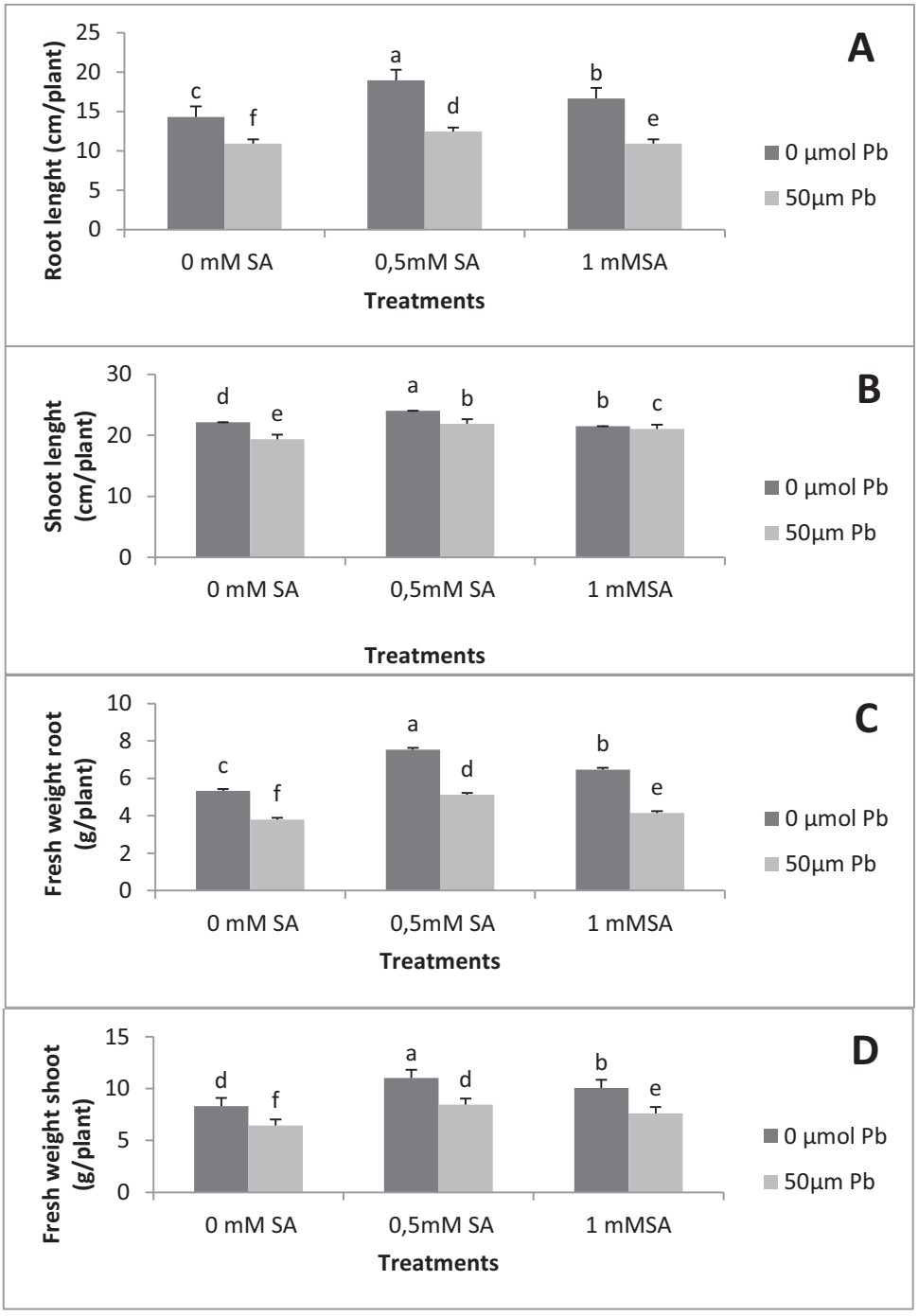

FIGURE 1

Effect of salicylic acid on the Root Length (A), Shoot Length(B), Fresh Weight Root (C) and Fresh Weight Shoot (D)of Faba bean (*Vicia faba*.L).Plantswere grown under lead stress condition. Error bars = Standard error of means (S.E.M.), Different letters superscript indicate a significant difference at $p \leq 0.05$.

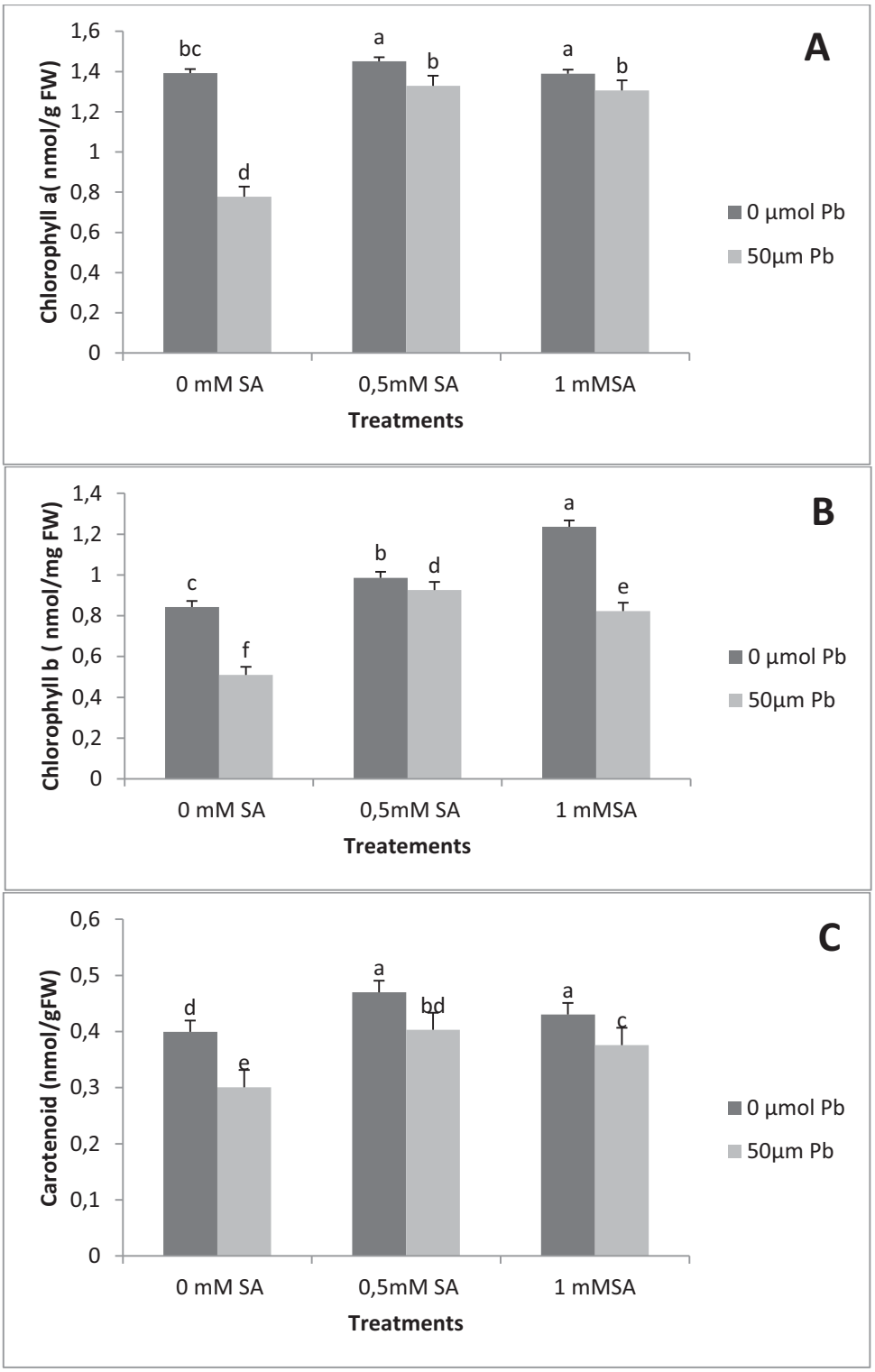

FIGURE 2

Effect of salicylic acid on chlorophyll a (A), chlorophyll b (B) and carotenoids (C) of Faba bean (*Vicia faba*.L). Plants were grown under lead stress condition. Error bars = Standard error of means (S.E.M.), Different letters superscript indicate a significant difference at $p \leq 0.05$.

RESULTS

Growth parameters. Lead significantly reduced all the detected growth parameters in the faba bean (*Vicia faba* L.) compared as compared to control plants (Figure 1). However, SA treatment ameliorated the growth parameters. The combination of Pb stress with SA led to a significant growth improvement compared to the Pb treatment alone. This increase remained important for the plants treated with 0.5 mM SA, which demonstrated an increases

30.27%, 26.56%, 33.85%, 29.89%, for the root length, shoot length, fresh weight root and fresh weight shoot respectively compared to the Pb plants.

Photosynthetic pigments. Lead stress affected the content of photosynthetic pigments in Vicia faba *L*.Chlorophyll a,Chlorophyll b and carotenoid contents, which were decreased by 52.16%, 19.53%,and 20.45% under Pb toxicity (Figure 2A,B,C). The combination of pb stress with SA treatment at 0.5 mM led a significant improvement in the content of all photosynthetic pigments parameters compared to Pb application.

Malondialdehyde(MDA) and Hydrogen peroxide (H_2O_2) contents. Under Pb stress, the accumulation of MDA and H_2O_2 were increased in the leaves of plants (Figure 3 A, B), However, application of SA (0.5 and 1mM) inhibited the accumulation of MDA andH_2O_2 and the effects were augmented more significant with 1mM SA than 0.5 mM SA .Application of 0.5mM SA and 1mM SA reduced MDA by 17.45% , 35.84% andH_2O_2by25.72% , 38.94%respectively, compared to Pb application.

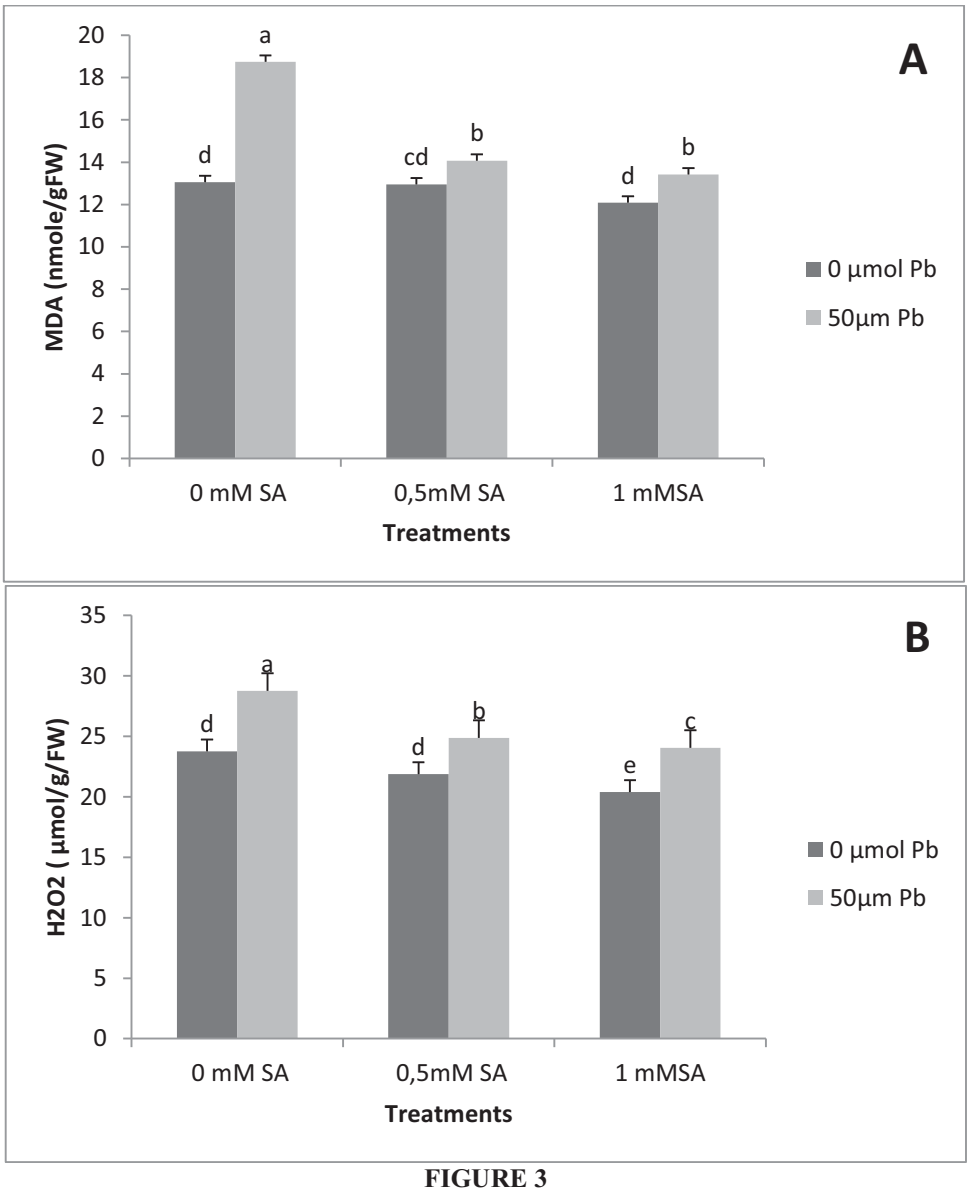

FIGURE 3

Effect of salicylic acid on MDA content (A) and H_2O_2 content (B) of Faba bean (*Vicia faba*.L). Plants were grown under lead stress condition. Error bars = Standard error of means (S.E.M.), Different letters indicate a significant difference at $p \leq 0.05$.

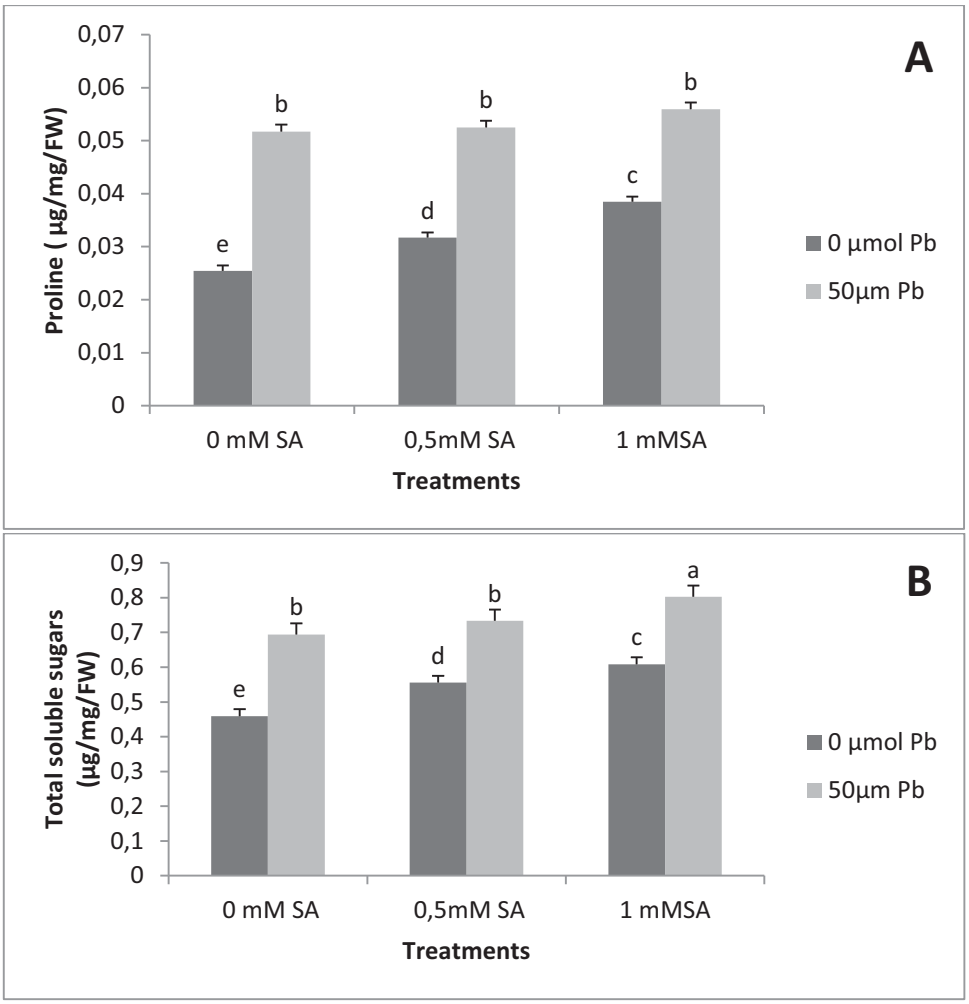

FIGURE 4

Effect of salicylic acid on proline content(A) and total soluble sugars content(B) of Faba bean (*Vicia faba*.L). Plants were grown under lead stress condition. Error bars = Standard error of means (S.E.M.), Different letters indicate a significant difference at $p \leq 0.05$.

Osmolytes Contents. Under unstressed conditions, the accumulation of osmolytescontents (Proline and total soluble sugars) were increased depending to the levels of SA (Figure 4 A,B). In other words, application of 0.5mM and 1mM SA increased Pro (48.99%, 85.83%) and TSC (27.65%, 46.36%) respectively, compared to control plants. Meanwhile, under Pb stress, plants showed higher levels of proline and total soluble sugars. The addition of SA further increased proline and total soluble sugars. In other words, application of 0.5 mM and 1mM SA enhanced the levels of proline by 12.4% and 22.51% and total soluble sugars by 15.06% and 19.58% respectively as compared to Pb alone.

Antioxidant Enzymes Activity. Under unstressed conditions, the antioxidant enzymes activities (CAT and APx) were enhanced according to the levels of SA (Figure 5 A,B)). In other words, Application of 0.5 mM and 1mM SA increased the activity of APx by (18.03%, 25.25%) and CAT by (20.79%, 37.23%) respectively, compared to control plants. Pb stress in plants enhanced the activity of antioxidant enzymes and the application of AS enhanced antioxidant enzymes activities further. The variations were most notable with 1mM SA for all enzymes activities. In that case the activity of APx was risen by (21,45%, 42,95%)and the activity of CAT was augmented by (32.25%, 40 ,97%) respectively, in plants either treated with 0,5 mM or 1mM SA, compared to plants treated with Pb separately.

DISCUSSION

Lead (Pb) is an example of a hazardous heavy metal, which it can be easily absorbed and accumulated in different plant tissues and organs [32]. The findings indicated that under Pb stress, all the detected growth parameters were significantly reduced in the faba bean (*Vicia faba* L.) compared to the untreated control plants.These findings are similar to those documented in several plant species, such as wheat [33]., Kidney Bean [34]., and mustard plants [35]. This reduction in growth could be attributed in part to the obstruction in nutrient uptake from the

roots due to interference of absorbed Pb with metabolic and biochemical processes associated with normal growth and development of the plants [36,37].

Application of SA improved growth parameters by mitigating adverse effects of Pb [38,39,40,41].SA induces cell division within the apical meristem of seedling resulting in increased plant growth [42]. In addition, application of SA might be beneficial in improving plant growth or in alleviating the toxic effects of Pb by restoring of hydraulic conductance and stimulating of antioxidant response.

Photosynthetic pigments are crucial components of photosynthesis and play important role in plant growth and the yield of dry matter.In this investigation, Pb stress resulted in a significant reduction of chlorophyll a, chlorophyll b, and carotenoid content in the leaves of faba bean plant. There is an explanation that Pb exerts a negative effect on photosynthesis by disrupting the ultrastructure of chloroplasts and preventing the synthesis of essential pigments, prevents the Calvin cycle and the electron transport chain, and induces a CO_2 deficiency by closing the stomatal pores [43]. The reduction in photosynthetic pigments under Pb stress might be also attributable to MDA accumulation or it probably due to the photosynthesis inhibition and the interaction of Pb with the sulfhydryl group in the enzymes involved in chlorophyll biosynthesis. Furthermore, Pb there by impeding the absorption of essential elements, such as Mg, Fe , or the substitution of divalent cations with this metal.

However, it was clear found that the application of SA improved the accumulation of photosynthetic pigments under Pb stress condition. SA plays an interesting role for reducing chlorophyll degradation by regulating antioxidant molecules synthesis, suppressing genes involved in the senescence process, up-regulating chlorophyll biosynthesis genes, and down-regulating chlorophyll degradation genes [44].

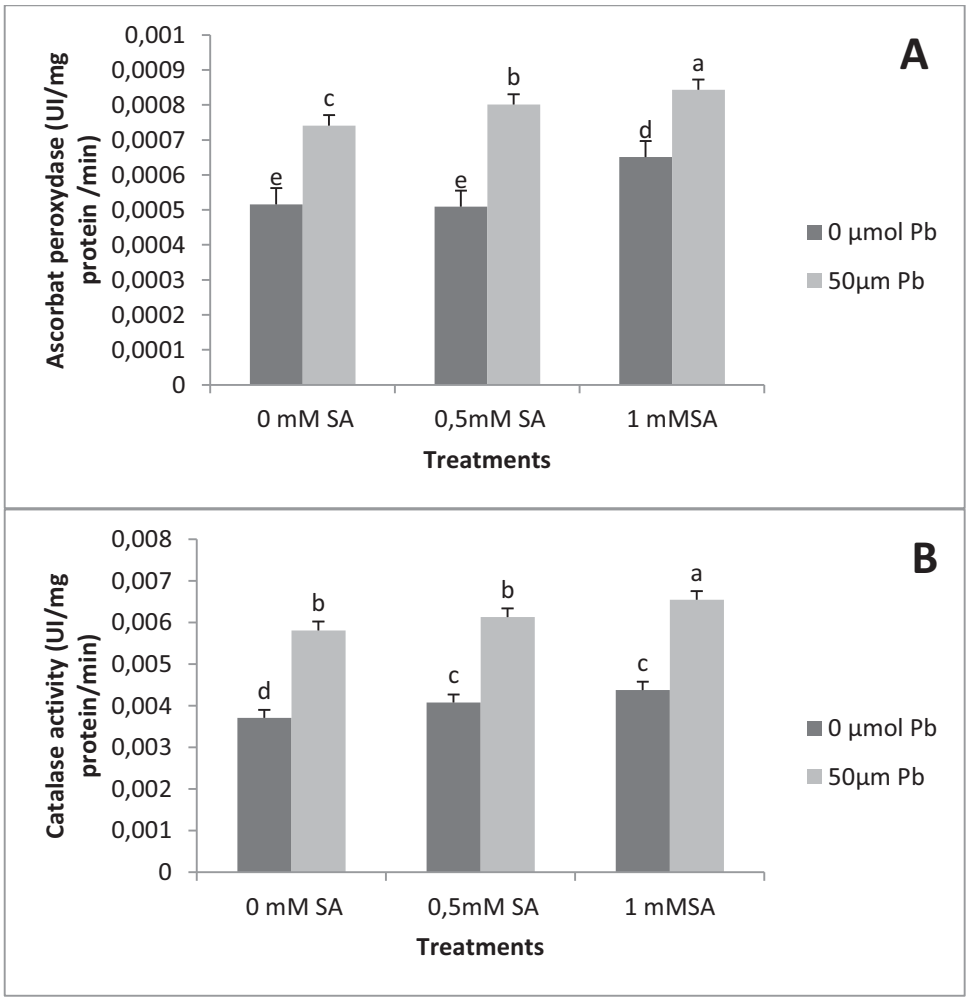

FIGURE 5

Effect of salicylic acid on ascorbate peroxidase (A) and catalase (B) activities of Faba bean(*Vicia faba*.L). Plants were grown under lead stress condition. Error bars = Standard error of means (S.E.M.), Different superscript letters indicate a significant difference at $p \leq 0.05$.

To cope with abiotic stress, plants accumulate certain compatible solutes whic are known as osmolytes such as soluble sugars and proline to protect the cellular machinery. The higher accumulation of proline under Pb stress was attributed to enhanced activities of proline biosynthesis enzymes,(ornithine aminotransferase and pyrroline-5-carboxylate reductase), as well as due to inhibition of proline degradation enzymes,(proline oxidase and proline dehydrogenase) [45]. Moreover, our study showed that SA stimulates proline accumulation and total soluble sugars in Pb stressed plants. Increasing the amount of proline and sugars in the plants would lead to resistance of leaf water loss and increase plant growth during stress . On the other hand, The accumulation of proline in the plant reduces damage to the membrane and proteins[46]. Proline, in addition to osmotic regulation, regulates cell pH, regulates oxidation and regeneration, and considers as a source of carbon and reduced nitrogen.[47].

The oxidative reactions induce a series of reactions that it lead to membrane peroxidation. The faba bean plants in this experiment had a high MDA and H_2O_2levels under Pb-stress treatment, which could result in the breakdown of plant cells under the ROS directly attack the biomolecules and polyunsaturated fatty acids including linoleic and lenolenic. Then, it forms complex mixture of lipid hydroperoxides and decreases the permeability of the membranes. The results confirmed the previous findings that Pb toxicity in plants increases lipid peroxidation and H_2O_2 production [48]. However, SA treatment inhibited MDA accumulation and H_2O_2, which it might be due to increased levels of proline, the accumulation of total soluble sugar, and increased antioxidant enzymes activities, as these inhibit ROS overproduction [49,33].

Plants have a defensive system of antioxidants to protect against ROS. These antioxidants play a pivotal role in the alleviation of abiotic stresses. For instance, CAT is a heme-containing enzyme and reduces the overproduction of H_2O_2 under oxidative stress through converting H_2O_2 into H_2O , whereas APX, the first enzyme of the AsA-GSH cycle, catalyzes H_2O_2 by utilizing AsA as an electron donor [50]. The findings indicated that CAT and APX activities increased when faba bean plants were exposed to Pb stress.In that case, the increased activity of these enzymes caused a tolerance rise of the faba bean plant to Pb by modulating the relative quantities of ROS. Interestingly, application of SA further increased these enzymes activities under Pb toxicity. These results might be related to the binding of SA with enzyme protein. Alamri et al. (2018) reported that SA can directly scavenge generated ROS and/or indirectly induce the redox potential of antioxidant enzymes.

In conclusion, Pb stress affected growth, pigment contents and other physiobiochemical attributes and antioxidant responsesof faba bean (*Vicia faba*L.). Whereas, application of SA solution contributed to a reduction of the injurious effect of Pb on the previous parameters. Thus, the protective role of SA mainly includes the stimulation of antioxidant enzyme activities,reducing the level of lipid peroxidation and regulation of osmotic adjustment through the accumulation of osmotic solutes (Proline, total soluble sugars) in Pb-stressed plants. It was also found that the effectiveness of SA in inducing Pb stress tolerance depends upon the concentration of SA supplemented.

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