

Effect of purified wastewater from the city of Settat (Morocco) on the quality of spearmint essential oil (*Mentha Spicata*)

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Abstract— *Mentha Spicata* is an aromatic plant largely consumed in Morocco. It is well-known for its use in the food field, medicinal and aromatic. The production of this plant is for the local market and for export.

In some situations, the farmer may have to use wastewater for irrigation, this may cause harm to the consumer and the environment, given that the therapeutic activity of these plants or their extracts depends mainly on the quality of finished products.

The objective of this work is to study the influence of treated wastewater quality essential oils of some aromatic plants knowing that the effectiveness of the operation of the plant or its extract depends on the quality of the finished product. The essential oil composition was also affected and a disappearance of many in spearmint essential oil constituents was observed with an increase of 57,11% for Carvone, the major compound of spearmint essential oil.

Keywords; wastewater, Essential oil composition, Spearmint.

I. INTRODUCTION

Volume of water used in the world has grown more than twice the rate of population growth, and a growing number of regions reached the limit beyond which it is no longer possible to provide reliable water (for different uses) [1]

Agricultural reuse of wastewater is a process of increasingly integrated into policy management of water resources, even in industrialized countries and emerging economies, as evidenced by studies in Spain [2], Italy [3] and China [4].

*Mentha spicata*L. (Spearmint) is a creeping rhizomatous, glabrous and perennial herb with a strong aromatic odor. The oil of *M. spicata* is rich in carvone and presents a characteristic spearmint odor [5]. The species has been found useful as digestive and gastro-stimulant this is eaten in the form of chutney. Leaves are popularly used as tea flavouring agent, while herbalist use whole plant as carmi-native [6]. The fresh and dried plants and their essential oils are widely used in food, cosmetic, confectionary, chewing gum, toothpaste and pharmaceutical industries [7]. The essential oil of *M. spicata* showed strong insecticidal and mutagenic activity [8]

For centuries, man has always taken care of by plants, empirically, guided by tradition or custom. Most of the great doctors of the past were herbalists [9].

Currently, herbs have a considerable advantage thanks to the discovery of applications from their essential oils in health care and their uses in other areas of economic interest. Their uses are many they know demand increasingly high in global markets [10].

Knowledge of essential oil chemotypes and their behavior is fundamental because it allows consideration of the pharmacological activity as predictive pharmacokinetic and bioavailability.

For the same botanical species, the chemical composition of the essential oil is not immutable. Essential oils are produced by aromatic plants in the secretory cells. Their preparation is totally dependent on solar radiation in the absence of which the yield of aromatic nature are affected. In his presence, especially in terms of the presence of any radiation, types of components may vary considerably within a species [11].

The principle of hydrodistillation is that the distillation of binary mixtures immiscible. It consists in immersing the plant biomass in a still filled with water, which is then heated to boiling. The steam and gas released from the plant material form an immiscible blend. Components of such a mixture behave as if each were alone at the mixture temperature, ie the partial pressure of the vapor component is equal to the vapor pressure of the pure substance. This method is simple in principle and does not require expensive equipment [12].

II. MATERIALS AND METHODS

A. Framework and population of Settat city

City of Settat, located on the road Casablanca-Marrakech 72 km south of Casablanca, is the urban core of the province. The latter, with 9888.4 km², is characterized by its agricultural and industrial development is very important. The industrial area of the city Renfer me 44 units covering different fields.

The city's population grew from 96,217 inhabitants in 1994, 18,555 households, 116,570 in 2004, 24,303 households, of which 95% are supplied with drinking water by the local authority (RADEEC) from the dam Daourat, Ain N'zagh and occasional slick Sidi Al Aidi and are connected to the sewerage [13].

B. Plant material

Plantation was in May 2009 in experimental plots located in the Faculty of Science and Technics of Settat-Morocco (FSTS). Irrigation is done with the urban wastewater lagoons purified by the city of Settat-Morocco and water wells located in the FSTS considered as a witness.

Harvests were made by hand, the plants were cut 15-30 cm above the ground.

The quality of essential oils was also determined using a fresh leaves of spearmint harvested.

C. Extraction of Mentha essential oil

Distillation apparatus consisted of a heating cap, a 1.5 l extraction flask, a cooling system and a receiver for hydro distillate. Thirty grams of fresh plant leaves and 800ml of water were used and the distillation was carried out for 3 h after the mixture reached boiling at 100 °C. Hydrodistillation repetitions were done at least in duplicate depending on the spearmint leaves availability. The essential oil obtained was dried under anhydrous sodium sulfate and stored at 4 °C in the dark before analysis. Yield of essential oil is derived from the dried leaves; Table III presents a comparison of the yields of the two essential oils.

D. Water quality irrigation

The physico-chemical parameters (temperature, pH and conductivity) were measured in situ by means of a mercury thermometer (1/10°C), portable pH meter lutron (pH-206) and conductivity meter HANNA (HI-8733 with a margin of error of 2% (Tab. Annexe).

In the laboratory, the waters were analyzed for major ions (Ca⁺⁺, Mg⁺⁺, NH₄⁺, Cl⁻, SO₄⁻ and HCO₃⁻) and the nutritive salts. Chlorides and alkalinity were determined by titration (Appendix Table); Rodier 1996).

Analyzes of metallic trace elements were carried out by the technique of ICP.

E. Gas Chromatography-mass spectrometry

Essential oil was characterized using a gas chromatograph Trace GC Ultra equipped with autoinjector (Triplus) directly interfaced with a mass spectrophotometer with flame ionization detector (Pdains Q). Capillary column was VB-5 (5% of diphenyl and 95% of dimethylpolysiloxane), 30m length, 0.25mm i.d. and 0.25mm thickness. Separation conditions were: 25 °C for 2min, 25–180 °C at 4 °C/min and 180–300 °C at 40°C/min. Temperature of the injector was 220 °C. The volume injected was 1 µL. The carrier gas was helium with a

flow rate of 1.4mlmin⁻¹. The oil constituents were identified by comparison of their retention indices and their mass spectra with those of authentic samples. Quantitative analysis (in percent) was performed by peak area measurement. The following table II shows the major components of essential oils of spearmint (*Mentha Spicata*).

III. RESULTS AND DISCUSSIONS

1) Physicochemical analysis of irrigation water

Throughout the experiment, the chemical composition of these two sources of irrigation water has been determined and is detailed in Table 1.

The gravity irrigation planting is done 1 day / 3 source and water were analyzed at each irrigation.

TABLE I. Characterization of well water and wastewater purified by lagoons used for irrigation in the study.

Parameter	Well water		Wastewater purified	
	average value	Norm ^(*)	average value	Norm ^(**)
pH	7,11	6,5-8,5	7,63	6,5-8,4
Temperature (°C)	22	-	29,36	35
Conductivity (20°C) (µs/cm)	995	2700	1313,64	1200
BOD ₅ (mgO ₂ /l)	11,7	70	27	-
COD (mgO ₂ /l)	376,5	25	656,36	-
NH ₄ ⁺ (mg/l)	0,319	0,5	11,25	-
Cl ⁻ (mg/l)	437,36	750	850,7	350
Ca ²⁺ (mg/l)	26,32	100	97,94	-
Mg ²⁺ (mg/l)	12,66	100	53,63	-
HCO ₃ ⁻ (mg/l)	41,98	-	67,65	512
N-NO ₂ ⁻ (mg/l)	0,026	0,1	0,09	30
PO ₄ ³⁻ (mg/l)	0,57	0,7	3,27	-
SO ₄ ²⁻ (mg/l)	44,21	200	124,00	350
Suspended matter (mg/l)	-	-	130,4	200
Cd (mg/l)	≤0.002	0.005	≤0.002	0,01
Cr (mg/l)	≤0.002	0.05	0.004	1
Cu (mg/l)	0.004	-	0,0075	0,2
Ni (mg/l)	≤0.002	-	0,012	2
Zn (mg/l)	0.889	5	0,089	2
Hg (mg/l)	≤0.01	1	≤0.01	0,001

(*): MOROCCAN NORMS, 2002. Official Bulletin n° 5062 of 30 Ramadan 1423 setting standards for drinking water for human consumption.

(**): Standards of water quality for irrigation S.E.E-2007-Morocco (Secretariat of State to the Minister of Energy, Mines, Water and Environment, responsible for Environment)

Water wells physico-chemical analyzes showed that the COD exceeds the standard, it is loaded by Cl⁻ and PO₄³⁻ to the city of Settat is located on phosphate rock.

Wastewater physico-chemical analyzes showed that the COD is high and the concentration of Cl⁻ exceeds the standard because urban water is responsible for by chloride ions.

Other physico-chemical parameters meet the standards as well as metallic trace elements.

2) Chemical composition of *Mentha spicata* essential oil

The GC-MS analyzes of the essential oils of the *Mentha spicata* irrigated by two sources of water are present in the table II.

TABLE II. Effect of urban wastewater from the city of Settat (Morocco) purified by lagoon on the major components contained in the fresh leaves of *Mentha Spicata*.

Compounds	Retention time	a	b
α-Pinene	8.59	0,76	0,69
Camphene	9.06	0,20	Trace
3-Carene	10.04	1,33	1,01
β-Phellandrene	10.67	0,81	0,60
Limonene	12.01	31,13	27,77
1,8-Cineole	14.59	0,25	0,28
Cis-Ocimene	15.88	0,29	Trace
Borneol	16.85	0,56	0,84
Sabinene hydrate	17.28	Trace	0,39
Isocyclocitral	17.94	0,46	1,71
Trans-Carveol	18.87	3,90	1,79
Pulegone	19.42	0,33	0,30
Carvone	19.74	53,69	57,11
Bornyl acetate	21.63	0,26	0,27
iso-Limonene	22.79	0,22	trace
β-Bourbonene	24.32	0,68	0,71
Caryophyllene	25.12	0,40	Absent
Aromandrene	25.40	0,67	0,50
Germacrene D	27.31	0,87	0,65
Calamenene	27.78	0,22	0,22

a: plots irrigated with well water of the FST-settat

b: plots irrigated by wastewater from the city of Settat treated lagoon.

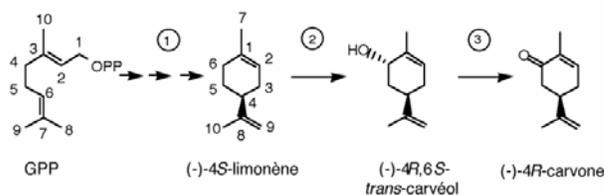
Mentha spicata of Morocco still contains majority composed: Carvone, Limonene and Trans-carveol. In general, the spearmint oil composition in this study was similar to that in other reports [17, 18].

When irrigation is the wastewater of the city of Settat we observe some modification composed of essential oils including majority composed such as carvone and Limonene, this is explained by Figure 1.

Geranyl diphosphate (GPP) is the physiological precursor of monoterpenes universal. Is cyclized from tertiary allylic isomer thereof, the (-)-3 R or (+)-3 S linalyl diphosphate according stereospecificity of enzymes. Synthetases produce monoterpenes from GPP a variety of monoterpenes. The reactions catalyzed by these enzymes are regiospecific with a moderate degree of enantioselectivity. Secretory trichomes of Lamiaceae have been a good starting material for the purification of these enzymes. Purification of the (4S)-limonene synthase, the first to be sequenced, resulted in the production of antibodies that can be used for any immunocytology subcellular localization.

biosynthesis via carvone and limonene from spearmint goes through two stages (figure 1) : le (-)-4S-limonene is hydroxylated on carbon 6 by the enzyme cytochrome monooxygenase selective, the 4S-limonene-6-hydroxylase that regenerates the (-)-4 R, 6S-trans-carveol. The latter is converted to (-)-4 R-carvone after deprotonation ensured by the (-)-trans-carveol dehydrogenase. These transformations are key steps in the biosynthesis of oxygenated monoterpenes in mints and the enzymes involved are completely regiospecific and exhibit a moderate degree of enantioselectivity. [14,15,16]

Figure 1. Biosynthetic pathway in spearmint carvone via limonene



(1): 4 S- LIMONENE SYNTHASE

(2): 4 S -limonene-6-hydroxylase

(3): (-)- trans -carveol dehydrogenase

This result is similar to results obtained by irrigation of spearmint by olive mill wastewater on spearmint [18].

3) Essential oil yields

Essential oils yield is determined by hydrodistillation of dried leaves on two plots.

TABLE III. Effect of urban wastewater from the city of Settat (Morocco) purified by lagoon on essential oil yield from dried leaves of *Mentha Spicata*.

	% Essential oil yield from dried leaves
(1)	0.54 ± 0.03
(2)	0.58 ± 0.03

- (1): plots irrigated with well water of the FST-settat
- (2): plots irrigated by wastewater from the city of Settat treated lagoon.

From these results we see that the urban wastewater lagoons purified by the city of Settat-Morocco has a beneficial effect and may increase essential oils yield.

IV. CONCLUSION

The results obtained by the irrigation waters urban city of Settat (Morocco) purified by lagoons promote the transformation of Limonene (cyclic hydrocarbon) in Carvone (cyclic ketone) and therefore play the role of two enzymes: 4 S-limonene-6-hydroxylase and (-) - trans-carveol dehydrogenase.

Carvone is the major compound of spearmint essential oil. It is widely decisive for the value of the essential oil, for fragrance and pharmaceutical industries. Carvone is an oxygenated monocyclic ketone highly odoriferous and valuable, accumulated in spearmint glandular trichomes specially [19]. Concentration of carvone in spearmint is usually above 50%, with the more desirable range being between 60% and 70% [20 and 21].

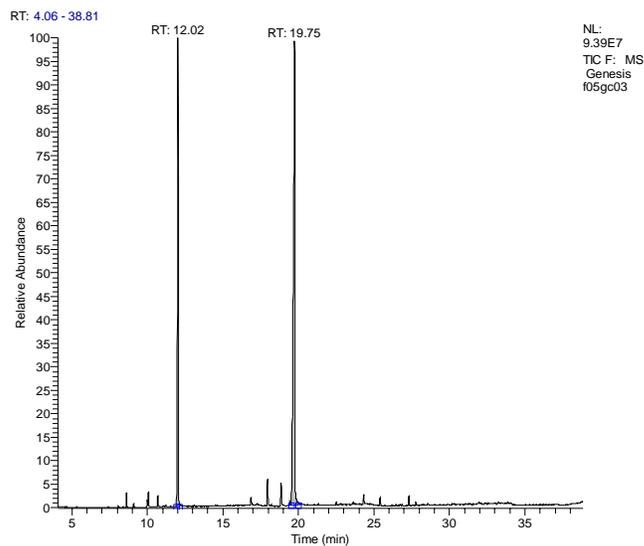
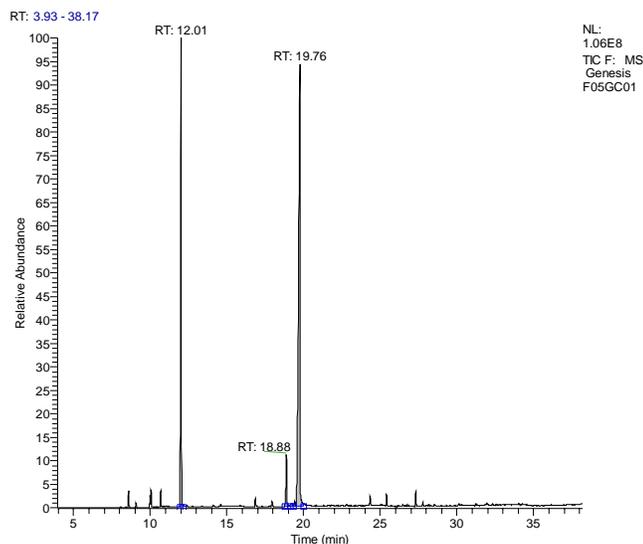
These wastewaters have a beneficial effect on yield and can increase the amount of spearmint essential oil: Decrease of Limonene 31,13 % at 27,77% and Trans-carveol of 3,90 % at 1,79 % and increase of Carvone 53,69% at 57,11%.

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APPENDIX:

Comparison of GC/MS profiles of the spearmint essential oil obtained in control assay (F05GC01) field receiving wastewater from the city of Settat (Morocco) treated lagoon (F05GC03) and RT = retention time.



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Annex Table - methods used in water analysis (Rodier 1996)

Parameters	Methods of analysis parameters
pH	Electrometry, Norm NF T 90-008
T°	Thermometry, Norm NF T 90-100
Conductivity (20 ° C)	Electrometry, Norm NF T 90-031
N-NO ₂ ⁻	Spectrometry, Norm NF T 90-013
NH ₄ ⁺	Spectrometry, Norm NF T 90-015
BOD ₅	Norm NF T 90 – 103
COD	Norm NF T 90 – 101
SO ₄ ²⁻	Nephelometry, Norm NF T 90-009
Orthophosphates	Norm NF T 90 – 023
Chlorures	Mohr method, NF T 900 – 014
Hardness (Ca ²⁺ , Mg ²⁺)	Volumetry, Norm NF T 90 – 003