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REVIEW ARTICLE

Effect of purified wastewater from the city of Settat (Morocco) on the quality of *Lippia citriodora* essential oil and infusion

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KEYWORDS

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Essential oil;
Wastewater;
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Neral and geranial

Abstract *Lippia citriodora* is an aromatic plant largely consumed in Morocco and throughout the world. It is well-known for its use in the field of food, medicine and aroma therapy. The production of this plant is for its local uses and for export. The therapeutic activity of *L. citriodora* or itsextracts mainly depends on the quality of finished products.

In this study, we aimed to evaluate the effect of irrigation with urban wastewater from Settat (Morocco) treated by lagoons on the composition of the essential oil, essential oil yield and physico-chemical composition infusion of *L. citriodora.*

The soil used for the implantation is characterized. Physico-chemical analyses of well water and wastewater used for irrigation are analyzed.

From the results obtained we found changes in the composition of the essential oil of a large number of constituents. After irrigation of *L. citriodora* by wastewater we observe changes in major compounds in the essential oil: decreased neral (from 15.29% to 14.34%) and geranial (from 15.63% to 14.75%) and increased limonene (from 23.39% to 25.86%). We deduce that wastewater has a beneficial effect in increasing the yield of essential oil.

The infusion of *L. citriodora* prepared by the fresh and dried leaves irrigated with wastewater contains a high concentration of NH_4^+, Ca^{2+}, Mg^{2+}, and the concentration of NO_2–N (mg/L) exceeds 100% of the concentration of waste water.

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1. 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) (FAO (2007). Agricultural reuse of wastewater is a process increasingly integrated into the policy management of water resources, even in industrialized countries and emerging economies, as evidenced by studies in Spain (Cazurra, 2008), Italy (Lopez and Vurro, 2008) and China (Wang et al., 2007).The genus *Lippia* (Verbenaceae) includes approximately 200 species of herbs, shrubs and small trees (Terblanché and Kornelius, 1996).

The lemon verbena, *Aloysia triphylla* (L'Herit.), Britt's = *Lippia citriodora* (Lam.), grows spontaneously in South America and is cultivated in North Africa (Morocco) and Southern Europe. In these areas, the leaves are largely used as herbal tea for their aromatic, digestive and antispasmodic properties. The lemon verbena is a folk remedy for colds, fever, spasms asthma, flatulence, colic, diarrhea, indigestion, insomnia and anxiety (Duke, 1985; Bézanger-Beauquesne et al., 1990; Carnat et al., 1999). The essential oil from its leaves has been shown to exhibit antimicrobial activity (Duarte et al., 2005; Duschatzky et al., 2004; López et al., 2004; Ohno et al., 2003; Sartoratto et al., 2004).A number of publications deal with the analysis and identification of the phenolic compounds (flavonoids and phenolicacids) of the leaves of *L. citriodora* (Carnat et al., 1995, 1999; Nakamura et al., 1997; Skaltsa and Shammas, 1988; Tomas-Barberán et al., 1987; Valentão et al., 1999).The chemical composition of the essential oil from the leaves of *L. citriodora* has also been studied and reviewed (Bellakhdar et al., 1994; Carnat et al., 1999; Catalan and de Lampasona, 2002; Crabas et al., 2003; Kim and Lee, 2004; Montes et al., 1973; Özek et al., 1996; Pascual et al., 2001; Santos-Gomes et al., 2005; Sartoratto et al., 2004; Terblanché and Kornelius, 1996; Velasco-Negueruela et al., 1993; Von Kaiser and Lamparsky, 1976a,b; Zygadlo et al., 1994). The genus *Lippia* shows a rich genetic diversity, enabling it to synthesize a variety of essential oil constituents in plants grown in different parts of the world (Catalan and de Lampasona, 2002; Santos-Gomes et al., 2005). However, the composition of the essential oil obtained from the same plant stock remains constant under the same environmental conditions (Catalan and de Lampasona, 2002; Santos-Gomes et al., 2005).

Perennial aromatic plants are cultivated as cash-crops for fresh or dry herb production, or as a source of essential oils and natural antioxidants. These summer crops require substantial amounts of water, up to 7000–9000 m^3 ha^{-1} throughout the growing season, to satisfy their potential for intensive biomass production (Putievsky et al., 1990; Dudai, 2005).

Hundreds of hectares of these crops are required to facilitate an economically viable industrial production system. Therefore, shortage of fresh water for irrigation in arid and semiarid regions restricts utilization of aromatic plants as industrial crops. Replacement of fresh water with treated effluent for irrigation of these plants could promote the development of large-scale production systems for biomass, essential oil, and natural antioxidants in arid and semiarid zones. Cultivation of aromatic plants for essential oils is suitable for irrigation with treated effluents because the heat applied during oil extraction eliminates human bacterial pathogens originating in the effluents and alleviates health concerns. Additionally, the essential oil, which is extracted mainly by steam distillation, will be free of inorganic ion contaminants such as heavy metals originating from the effluents, which may accumulate in the plant tissues and the soil (Nirit Bernstein et al., 2009).

2. Materials and methods

2.1. Framework and population of Settat city

Settat City, located on the road Casablanca–Marrakech 72 km south of Casablanca, is the urban core of the province. This latter, with 9888.4 km^2, is characterized by its agricultural and industrial development very important. The industrial area of the city renferme consists of 44 units covering different fields. The city's population grew from 96,217 inhabitants in 1994, 18,555 households, to 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 sewerage disposal(Statistics Directorate 2004. National Statistical Yearbook. Report of Population Census, 500 p).

2.2. Plant material

Plantation was established in May 2009 at experimental plots located in the Faculty of Sciences and Techniques of Settat-Morocco (FSTS). The irrigations were made by urban wastewater purified by lagoon from the Settat city (Morocco) and well water located in FSTS considered as a witness. The quality of essential oils was also determined using harvested fresh and dried leaves of *L. citriodora*.

Table 1 Characterization of the soil.

Parameter	Average value
pH	8.05
Conductivity (20 °C) (μs/cm)	1400
Nitrate-nitrogen (NO_3–N) (mg/L)	17.66
Phosphorus (P) (mg/L)	8.02
Potassium (K) (mg/L)	430
Organic matter (%)	3.5%

2.3. Planting soil

Before implantation an analysis of representative soil of the study area was performed. The soil is collected in Zigzag manner with a helical auger to depths of 20 cm. Sample types of the same depth are mixed, put in a plastic bag and transported to the laboratory. The soils were dried at 40 °C for three days, crushed using a porcelain mortar and sieved to 2 mm and bagged for various analyses. Table 1 presents the soil analysis before implantation. Measured soil pH (Mckeague, 1978; Mclean, 1982), conductivity (Richards, 1954), nitrates (Sims and Jackson, 1971; Hadjidemetriou, 1982), phosphorus (Olsen et al., 1954), potassium (Ammonium Acetate Extraction) and organic matter (Walkley and Black, 1934; Walkley, 1947; FAO, 1974).

2.4. 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^{++}, NH_4^+, Cl^-, SO_4^- and HCO_3^-) and the nutritive salts. Chlorides and alkalinity were determined by titration (Tab. Annexe; Rodier, 1996). Analyses of heavy metals were carried out by the technique of ICP. Table 2 presents all the physico-chemical analyses of well water and wastewater purified by lagoons used for irrigation in the study.

Table 2 Characterization of well water and wastewater purified by lagoons used for irrigation in the study.

Parameter	Well water		Wastewater purified	
	Average value	Norm[a]	Average value	Norm[b]
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
OD_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
Pb (mg/L)	0.01	0.05	0.069	5
Al (mg/L)	0.0228	0.2	0.117	5
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

[a] MOROCCAN NORMS, 2002. Official Bulletin n° 5062 of 30 Ramadan 1423 setting standards for drinking water for human consumption.
[b] Standards of water quality for irrigation S.E.E.E-2007-Morocco (Secretariat of State to the Minister of Energy, Mines, Water and Environment, responsible for environment).

Table 3 Effect of urban wastewater from the city of Settat (Morocco) purified in lagoon on the major components contained in the fresh and dried leaves of *L. citriodora*.

Compounds	Retention time	A	B	C
α-Pinene	8.62	0.94	–	1.06
Sabinene	10.00	2.22	2.33	2.42
Limonene	11.99	23.39	25.86	28.32
Trans-ocimene	12.75	2.40	2.67	–
Nerol	17.77	1.64	1.50	1.40
Neral	19.55	15.29	14.34	13.85
Geranial	20.57	15.63	14.75	14.06
β-Caryophyllene	25.41	3.52	3.98	3.35
α-Curcumene	27.37	9.37	10.81	11.36
Nerylacetate	27.78	4.84	6.64	2.38
Copaene	28.28	2.60	3.42	2.38
α-Cadinene	28.59	1.52	–	–
Spathulenol	30.14	6.28	5.43	8.29
Caryophylleneoxide	30.29	6.37	4.23	7.07
ϒ-Cadinene	31.93	3.86	3.75	3.86

A: Fresh leaves from plots irrigated with well water of the FST-settat.
B: Fresh leaves from plots irrigated with wastewater from the city of Settat's treated lagoon.
C: Dried leaves from plots irrigated with wastewater from the city of Settat's treated lagoon.

Table 4 Effect of urban wastewater from the city of Settat (Morocco) purified by lagoon on the essential oil yield from dried leaves of *L. citriodora*.

	% Essential oil yield from dried leaves
(1)	0.87 ± 0.03
(2)	1.01 ± 0.03

(1) Plots irrigated with well water from the FST-Settat.
(2) Plots irrigated with wastewater from the city of Settat's treated lagoon.

2.5. Extraction of L. citriodora 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 plant leaves and 800 ml 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 *L. citriodora* 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 4 presents a comparison of the yields of the essential oils.

2.6. Gas chromatography–mass spectrometry

The essential oil was characterized using a gas chromatograph Trace GC Ultra equipped with an auto injector (Triplus) directly interfaced with a mass spectrophotometer with a flame ionization detector (Pdains Q). Capillary column was DB-5 (5% of diphenyl and 95% of dimethypolysiloxane), 30 m in length, 0.25 mm thickness. Separation conditions were: 50 °C for 2 min, 50–200 °C at 5 °C/min. Temperature of the injector was 220 °C. The volume injected was 0.1 μL. The carrier gas was helium with a flow rate of 1.4 ml min^{-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. Table 3 shows the major components of essential oils of *L. citriodora*.

2.7. Infusion quality

Fresh leaves were collected which represent different plots of *L. citriodora* irrigated with urban wastewater from the city of Settat (Morocco) purified by lagoon. The collected samples were kept in plastic bags until their analysis.

The infusion was made by pouring 100 ml of boiling water on 10 g of plant material. The mixture was allowed to stand for 10 min, filtered and then used. The infusion was freshly prepared for each experiment. The leaves of *L. citriodora* were dried at room temperature. We followed the same procedures used for physico-chemical analysis of water (Table in Annexe). Table 5 presents all the physico-chemical analyses of infusion from fresh and dried leaves of *L. citriodora* irrigated with urban wastewater from the city of Settat (Morocco) purified by lagoon.

3. Results and discussion

3.1. Soil quality before implantation

Before starting the installation of conventional soil, analysis of the study area has been made.

Soil tests have shown that it is alkaline (pH = 8.05) moderately rich in nitrate, phosphorus and high potassium (pH = 8.05). This soil contains a higher percentage of organic matter (2%) containing nitrogen which is mineralized with time and it is a good soil for implantation.

3.2. Physico-chemical 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 2. The gravity irrigation is done 1 day/3, water is analyzed at each irrigation.

Table 5 Physicochemical characterization of infusion from fresh and dried leaves of *L. citriodora* irrigated with urban wastewater from the city of Settat (Morocco) purified in lagoon.

Parameter	A	B
	Average value	Average value
pH	6.85	6.37
Temperature (°C)	25	25
Conductivity (20 °C) (μs/cm)	1384	1265
BOD_5 (mgO_2/L)	28	30
COD (mgO_2/L)	1433.33	1033.33
NH_4^+ (mg/L)	59.913	61.780
Cl^- (mg/L)	284	639
Ca^{2+} (mg/L)	1231.26	1115.83
Mg^{2+} (mg/L)	734.75	656.03
HCO_3^- (mg/L)	87.84	197.64
N–NO_2^- (mg/L)	6.19	6.16
PO_4^{3-} (mg/L)	1.44	1.91
SO_4^{2-} (mg/L)	102.15	105.8
Al (mg/L)	0.335	0.153
Cd (mg/L)	$\leqslant$0.002	$\leqslant$0.002
Cr (mg/L)	0.005	0.002
Cu (mg/L)	0.266	0.103
Ni (mg/L)	0.040	0.181
Pb (mg/L)	0.078	0.098
Zn (mg/L)	0.659	0.493
Hg (mg/L)	$\leqslant$0.01	$\leqslant$0.01

A: Infusion prepared from fresh leaves of *L. citriodora* irrigated with urban wastewater purified in lagoons of Settat (Morocco).
B: Infusion prepared from dried leaves of *L. citriodora* irrigated with urban wastewater purified in lagoons of Settat (Morocco).

The physico-chemical analyses from well water showed that the COD exceeds the standard, it is loaded by ions Cl^- and PO_4^{3-}, this city of Settat is located on phosphate rock.

The physico-chemical analyses from wastewater showed that the COD is high and the concentration of Cl^- ions exceeds the standard because all urban waters are loaded with chloride ions.

Other physico-chemical parameters meet the standards as well as heavy metals.

3.3. Chemical composition of L. citriodora essential oil

The GC–MS analyses of the essential oils of the *L. citriodora* irrigated with two sources of water are present in Table 3.

Studies have mounted that *L. citriodora* always contains these major compounds: geranial, neral and limonene. Fig. 1. However, our results did not show the presence of p-cymene, camphor and thymol, which have been mentioned in other studies concerning *L. citriodora* (Kim and Lee, 2004; Zygadlo et al., 1994).

This result is similar to results obtained by (El Hassani et al., 2009) irrigation of spearmint by olive mill wastewater and (Bensabah et al., 2013) irrigation of spearmint by wastewater from the city of Settat's (Morocco) treated lagoon.

L. citriodora irrigated with wastewater brings modifications in components of the essential oils. There was an increase of limonene (Table 3). This is the opposite case of *Mentha spicata* irrigated with wastewater and olive water (El Hassani et al., 2009; Bensabah et al., 2013).

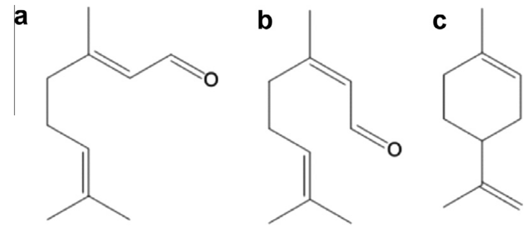

Figure 1 Molecular structures of geranial (a), neral (b) and limonene (c).

We recorded a decrease of neral and geranial and even the disappearance of the constituents of essential oils such as α-pinene, trans-ocimene and α-cadinene was observed (Table 3).

After irrigation of *L. citriodora* by wastewater we observe changes in major compounds in the essential oil: decreased neral (from 15.29% to 14.34%) and geranial (from 15.63% to 14.75%) and increased limonene (from 23.39% to 25.86%).

Drying *L. citriodora* had led to similar results as those of Djerrari, 1986 who noted qualitative and quantitative changes occurring in the essential oil of basil during drying in air at 25 °C. Indeed, after drying for three days, there was a quantitative reduction of the following volatile compounds: trans-ocimene, geraniol, geranial, eugenol, neryl acetate, humulene and Y-muurolene and compounds which increased quantitatively are: α-pinene, β-pinene, α-and β-copaene element.

These changes composed of the essential oils can be explained as follows:

From the GPP (geranyl diphosphate) (Croteau, 1987) or its isomer (NPP neryl diphosphate) (Ames and MacLeod, 1990), or even the LPP (Gleizes et al., 1982; Alonso and Croteau, 1991), the process of biosynthesis of acyclic monoterpenes as a whole through a series of carbocations is divided into a series of steps: ionization, or allylic transposition migration diphosphate group, turning bond, dehydrogenation and rearrangement of the cations. Current knowledge is summarized in Fig. 3. Geraniol and nerol are derived directly from the GPP by hydrolysis. Citronellol and linalool come from the GPP or LPP, one by reducing the other by hydrolysis transposed.

Other precursors of monoterpenes (Fig. 2) could be linalyl diphosphate (LPP) and terpenyl diphosphate (TPP) formed from GPP (Gambliel and Croteau, 1984; Pérez et al., 1990).

Aldehydes – geranial, neral, and citronellal – come from the corresponding alcohols by enzymatic oxidation. Direct precursors of ocimene and myrcene, acyclic hydrocarbons, and stages of formation are still unclear. Loomis and Croteau (1980) consider a 1,4-dehydration of geraniol. Gleizes et al. (1982) and Alonso and Croteau (1991) have shown that these compounds are derived from GPP or LPP allylic transposition after an electrophilic mechanism and rotation link.

3.4. Essential oil yields

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

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

Because of its wealth of nutrients and trace elements wastewater is reused for irrigation, leading to improved yields of crops. In this context, Fars et al. (2003) and Mohammad

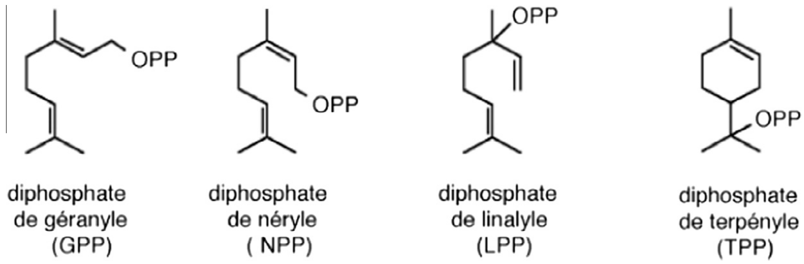

Figure 2 Structures of precursors of monoterpenes.

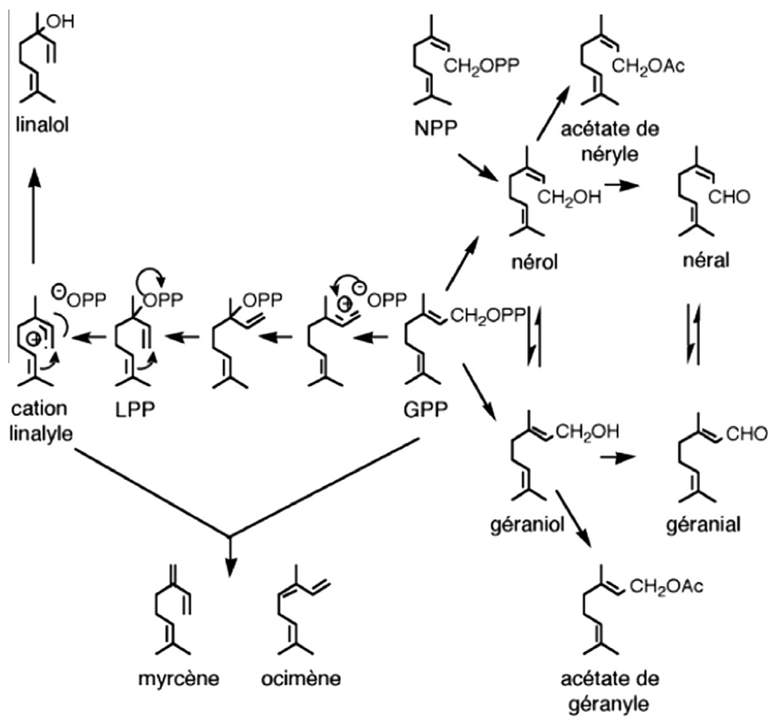

Figure 3 Biosynthetic steps leading to acyclic monoterpenes (Gleizes et al., 1982; Alonso and Croteau, 1991).

Rusan et al. (2007) found an increase in the biomass of forage when irrigated with wastewater or raw or processed. Thus, Fars et al. (2003) indicate that yields recorded over a witness exceed 110% for plants irrigated with these waters.

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

3.5. *Physicochemical analysis of infusion from fresh and dried leaves of L. citriodora irrigated with urban wastewater*

The chemical composition of infusion from fresh and dried leaves of *L. citriodora* irrigated with urban wastewater from the city of Settat (Morocco) purified by lagoon has been determined and is detailed in Table 5.

From these analyses we deduce that the infusion of *L. citriodora* prepared by the fresh leaves irrigated with wastewater contains the following percentages:

25.36% of NH_4^+, 59.86% of Ca^{2+}, 65.24% of Mg^{2+}, and 327.51% of N–NO_2^-. compared to concentrations from wastewater.

While this infusion contains:

1.59% of Cl^-, 6.18% of HCO_3^{-}·, 2.10% of PO_4^{3-}·, and 3.92% of SO_4^{2-}·.

And *L. citriodora* infusion prepared by dried leaves irrigated with wastewater contains the following percentages:

26.15% of NH_4^+, 54.25% of Ca^{2+}, 58.25% of Mg^{2+}, and 325.93% of N–NO_2^-compared to concentrations from wastewater.

While this infusion contains:

3.58% of Cl^- ,13.91% of HCO_3^-, 2.78% of PO_4^{3-}, and 4.06% of SO_4^{2-}compared to concentrations from wastewater.

The concentration of N–NO_2^- in the infusion that exceeds 100% of the wastewater could be explained by the mineralization of the existing organic matter in the soil.

The results of the analysis of heavy metals showed that all concentrations do not exceed 100%, except the concentration of Cu which is 168.89% of the concentration from wastewater. We can explain this result as the soil could also contain copper.

Mineralization is assimilation by soil organisms in organic matter as an energy source and as a component for their metabolism in which the OM is transformed into inorganic

(CO_2, N_2, etc.. . .). The mineralization rate is highly dependent on the nature of the MOS on the one hand and environmental factors such as soil aeration, nutrient content, temperature, pH, accessibility organisms of OM, the water content on the other (Davidson et al., 1995).

Indeed, irrigation with treated wastewater does not result in a systematic accumulation of OM in the soil and the reverse can even be observed. Indeed, due to its richness in nutrients and trace elements, the treated wastewater stimulates microbial activity in the soil (Magesan et al., 2000; Ramirez-Fuentes et al., 2002), promoting the mineralization of organic carbon in the ground when conditions are favorable, resulting in a lower rate of OM in the soil (Solis et al., 2005. Herpin et al., 2007).

4. Conclusion

This study found that urban wastewater treated by lagoon causes a beneficial effect on the yield of the essential oil of *L. citriodora*.

These wastewaters change the percentage of a large number of constituents of essential oils and especially the major compounds: decreased neral (from 15.29% to 14.34%) and geranial (from 15.63% to 14.75%) and increased limonene (from 23.39% to 25.86%).

On the contrary infusions have a harmful effect on health because we have found that the infusion of *L. citriodora* prepared by the fresh and dried leaves irrigated with wastewater contains high concentrations of NH_4^+, Ca^{2+}, and Mg^{2+} and the concentration of NO_2–N (mg/L) exceeds 100% of the concentration from wastewater.

Acknowledgments

We thank the CNRST (National Center of Scientific and Technical Research, Rabat, Morocco) for GC/MS and ICP analyses.

Appendix A. 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

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