Journal of ecology of Health & Environment
An International Journal

http://dx.doi.org/10.18576/jehe/050203

Chemical Structure of Natural Products and Characterization of Secretory Tissue of Sweet Basil (*Ocimumbasilicum L.*)Under Lead Stress

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Received: 2 Mar. 2017, Revised: 2 Apr. 2017, Accepted: 26 Apr.2017.
Published online: 1 May.2017.

Abstract: The present study was done to test the effect of Pb treatments on secretory tissue and the chemical structure of the essential oils of basil (*Ocimumbasilicum L.*). Lead treatments were as follow: 100, 250, 500 and 7500 ppm in addition to control. The experiment was done on *Ocimumbasilicum L.* in a greenhouse, arranged as a randomized complete block design with three replications for each treatment for 60 days.The morphological structure of the secretory tissue of basil leaves was investigated, using SEM (scanning electron microscope).The chemical composition of natural products of sweet basil exposed to lead treatments extracted by methanol and n-hexane, was analyzed by gas chromatography coupled with mass spectrometry (GC–MS). The Results revealed the presence of organic aldehyde, diterpenes, monoterpenes, and phenolic compounds in addition to unknown substances. Compounds in the hexane fraction included camphor, 1,8-Cineol, L-,alpha-Terpeniol, Eugenol, Methyl Eugenol, Beta Elemene, Pachoulene, gamma Cadinene, 9,12,15-octadecatrienoic acid,methyl ester, Delta Cadinene, Linolenic acid β- cadinene,stearic acid, and an unknown substances . Compounds in the methanol fraction included furfural, 1,8-Cineol, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, trans geraniol, β-Linalool,L-,alpha-Terpeniol, Alpha-Fenchyl acetate, Eugenol, Methyl Eugenol, 9,12,15-octadecatrienoic acid, methyl ester, gamma Cadinene, Linolenic acid, β-elemene, bicycle[3.1.1]hept-2-ene,2,6-dimethyl-6-[4-methyl-3-pentenyl]- and unknown substances. Results were concluded that *Ocimumbasilicum* is tolerant to lead treatments until 200 ppm, so it can be used as alternative crops for Pb enriched soils.

Keywords: Essential oils; Sweet Basil; Methyl eugenol; lead; Aromatic plants; Phytotoxin.

1 Introduction

Common basil (*Ocimumbasilicum L.*), a member of the Lamiaceae family, includes herbs and shrubs distributed in tropical and subtropical regions of Asia, Africa and the Americas. The genus Ocimumcomprises more than 150 species and is considered as one of the largest genera of the *Lamiaceae*family [1]. The most important species of Ocimumgenus is *O. basilicum*L.; this species, usually named common basil or sweet basil. Basil is an essential oil crop which has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries [2].*Ocimumbasilicum*is the commonly used for cookery, pharmaceutical and cosmetic purposes. Some of its oil components, such as 1,8-cineole, linalool and camphor, are known to be biologically active [3].Antiviral and antimicrobial activities of this plant have also been reported [4].Available literature data indicates that there is a great deal of diversity in growth characteristics and the composition of essential oil of the genus *Ocimum*. According to world health organization (WHO), greater than 80% of the total world's population depends on natural products in order to satisfy their primary health care needs. Knowledge of the chemical composition of medicinal plants is desirable because such information will be of value for the synthesis of complex chemical substances [4]. The metals i.e. As, Se, Cd, Hg, Pb, etc. are very toxic to humans and environment and plants are suggested as potential biosorbents for trace metals removal from the soil.

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As one of the most common heavy metal contamination in the environment, lead(Pb)pollution has been addressedin the literature because of its hight oxicity level sand adverse effects on organism and human health[5]. Lead (Pb) is recalcitrantin the environment and can cause damage to biota [6].

The main objective of the present study was to evaluate the effect of Pb on secretory tissue and essential oils of basil, characterize the chemical composition and the adaptive significance of exudates and study the possibility for using basil as aromatic plants as alternative crops for Pb enriched soils.

2 Materials and Methods

2.1 Physiological Assays

Seeds of basil (*Ocimumbasilicum*L.) were selected uniformly and disinfected with 5% hypochlorite for 1min. After germination, uniform seedlings were transferred into plastic pots containing sand. Plants were treated with different concentrations of $PbNO_3$ (100, 250, 500 and 750 ppm) and under controlled environment [7].The treatments were arranged basis on a randomized block design with three replications. The experiment was performed in the research greenhouse of the Faculty of Science of Sohag University (Egypt). After 60 days of treatment period, plants were harvested for chemical analysis using GC-MS and microscopy investigation using SEM.

2.2 Scanning Electron Microscopy (SEM)

To investigate the secretory tissue and morphological characteristics of basil leaf surface using SEM, leaf samples were prepared according to[8].

2.3 Extraction of Essential Oils

About 2 g of fresh material or optional respectively to the weight of the quantity of frozen or lyophilized material was ground in a mortar and pestle with 15 mL of n-hexane and methanol, then centrifuged at 4000g for 15 min and evaporated under reduced pressure until the volume of the residual extracts was approximately 2 ml, after which it was transferred to a minivial and dried under a stream of nitrogen [9].

2.4 Chemical Identification of Basil Essential Oil by GC-MS

The chemical constituents of each extract were analyzed using gas chromatography coupled with mass spectrometry (GC–MS).GC–MS was performed on Agilent 5975 GC/MSD system. A DB-5 MS UI stainless steel capillary column 30 m x 0.25 mm (1.0 µm film thickness), The column temperature was initially held at 60 °C for 1 min. and then programmed to 280 °C atarateof25 °C per minute. Mass unit conditions were as follows: Ion source 230 °C, ionization energy 70 eV and electron current 1455 µA. Helium was used as the carrier gas at 1 ml per minute. The injection temperature was 200 °C. The WILEY 275data base was used for automatic identification of GC–MS peaks and linear retention indices were compared with the published data [10].

3 Results and Discussion

Scanning Electron Micrographs (SEM) depicted the impact of lead on the secretory tissue and external morphology of basil leaf, i.e. the epidermal cells, nectar glands and stomata (Fig. 1). As it can clearly be demonstrated, stomatal areas were observed on the top of the outer protrusions, responsible for nectar secretion (A, B). In addition, the stomata can be observed at the level of other epidermal cells. SEM micrograph showed that the stomatal cells occurred at the level of other cells of the epidermis or slightly beneath the epidermis and there is no stomata were found on the flat part of the nectar glands (C). Nectar glands covering broad area of the leaf surface (B). Pollen grains occurred on the epidermal cells surface (D, E).According to previous studies[11], hairs and trichomes were observed in the epidermis covering the outgrowths of the stamens protected the nectar against evaporation in the flowers of the observed varieties of basil; these trichomes also made it impossible for insects unable to cross pollinate flowers to use the nectar.Scavroni[12],demonstrated that sweet basil can be cultivated as a good phytoremediator in polluted soil as it was capable of developing without accumulation of these metals in its tissue.According to [13], under stress of high concentrations of Cd, Pb and Cu, dry matter of peppermint, basil (*Ocimumbasilicum*L.) and dill (*Anethumgraveolens*L.) were not affected.

The GC–MS analysis of leaf samples of different treatments extracted with n-hexane exhibited the presence of 28 compounds (Table 1) with eleven distinct peaks (Fig. 2) of terpenoid compounds, phenolic compounds, and organic aldehydes. Compounds in the hexane fraction included camphor,1,8-Cineol, L-,alpha-Terpeniol, Eugenol, Methyl Eugenol, Beta Elemene, Pachoulene, gamma Cadinene, 9,12,15-octadecatrienoic acid,methyl ester, Delta Cadinene, Linolenic acid β- cadinene,stearic acid, and an unknown substances (Table 1).Analysis of leaves extract of different treatments with methanol by GC–MS exhibited the presence of 19 compounds (Table 2), of which 12 with distinct peaks (Fig. 3). These were furfural, 1,8-Cineol, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, trans geraniol, β-Linalool,L-,alpha-Terpeniol, Linolenic acid, β-elemene, bicycle [3.1.1] hept-2-ene,2,6-substances (Fig. 3).

The results recorded in table 1 revealed that most of separated components of the hexane fraction were oxygenated diterpenes, which may contributed to the

Table 1. Chemical composition of the floral essential Oil of *O. basilicum* L. with n-hexane extract.

legend	No.	compound	R-time	M.Wt	M. formula
	1	alpha-Pinene	5.377	136	$C_{10}H_{16}$
	2	m-Ethyl toluene	5.554	120	C_9H_{12}
	3	o-Ethyl toluene	5.583	120	C_9H_{12}
	4	1,2,3-trimethylbenzene	5.617	120	C_9H_{12}
	5	Beta phellandrene = Sabinene	5.680	136	$C_{10}H_{16}$
	6	2-Beta-Pinene	5.686	136	$C_{10}H_{16}$
	7	Mesitylene = S-trimethylbenzene	5.841	120	C_9H_{12}
a	8	camphene	6.098	136	$C_{10}H_{16}$
b	9	1,8-Cineol = Eucalyptol	6.178	154	$C_{10}H_{18}O$
	10	Benzene,2-ethyl-1,4-dimethyl-	6.270	134	$C_{10}H_{14}$
	11	CisSabinene hydrate	6.413	154	$C_{10}H_{18}O$
	12	Camphor	7.076	152	$C_{10}H_{16}O$
c	13	L- ,alpha-Terpeniol	7.288	154	$C_{10}H_{18}O$
	14	Palmitinic acid	7.776	256	$C_{16}H_{32}O_2$
	15	Alpha-Fenchyl acetate	7.854	196	$C_{12}H_{20}O_2$
	16	6-vinylspiro(2.4)hept-4-ene	8.232	120	C_9H_{12}
d	17	Eugenol	8.243	164	$C_{10}H_{12}O_2$
e	18	Methyl Eugenol	8.418	178	$C_{11}H_{14}O_2$
f	19	Beta Elemene	8.495	204	$C_{15}H_{24}$
	20	Cis beta Farnesene	8.684	204	$C_{15}H_{24}$
g	21	Pachoulene	8.713	204	$C_{15}H_{24}$
	22	Trans Caryophyllene	8.787	204	$C_{15}H_{24}$
	23	Alpha Humulene	8.993	204	$C_{15}H_{24}$
h	24	Gamma Muurolene = gamma Cadinene	9.234	204	$C_{15}H_{24}$
i	25	9,12,15-octadecatrienoic acid,methyl ester	9.794	292	$C_{19}H_{32}O_2$
j	26	Delta Cadinene	9.903	204	$C_{15}H_{24}$
k	27	Linolenic acid	10.172	278	$C_{18}H_{30}O_2$
	28	Octadecanoic acid = Stearic acid	10.321	284	$C_{18}H_{36}O_2$

Table 2. Chemical Composition of the floral essential oils of *O. basilicum* L. with methanol extract.

Legend	No.	compound	R-time	M.Wt	M. formula
a	1	Furfural	4.564	96	$C_5H_4O_2$
	2	Furfuryl alcohol	4.805	98	$C_5H_6O_2$
		Methyl-2-Furfural	5.577	110	$C_6H_6O_2$
	3	Benzyl alcohol	6.150	108	C_7H_8O
b	4	1,8-Cineol	6.172	154	$C_{10}H_{18}O$
		Trans-Sabinene hydrate	6.430	154	$C_{10}H_{18}O$
c		Beta-Linalool	6.510	154	$C_{10}H_{18}O$
d	5	L- ,alpha-Terpeniol	7.290	154	$C_{10}H_{18}O$
	6	5 Hydroxymetylfurfural	7.357	126	$C_7H_8O_3$
	7	Trans geraniol	7.506	154	$C_{10}H_{18}O$
	8	Palmitinic acid	7.752	256	$C_{16}H_{32}O_2$
e	9	Alpha-Fenchyl acetate = Bronyl acetate	7.854	196	$C_{12}H_{20}O_2$
	10	2 methox-4-vinylphenol	7.998	150	$C_9H_{10}O_2$
	11	2-oxabicyclo[2.2.2]octan-6-ol,1,3,3-trimethyl-,acetate	8.141	212	$C_{12}H_{20}O_3$
f	12	Eugenol	8.238	164	$C_{10}H_{12}O_2$
g	13	Methyl Eugenol	8.409	178	$C_{11}H_{14}O_2$
h	14	Beta-Elemene	8.496	204	$C_{15}H_{24}$
i	15	Bicycle[3.1.1]hept-2-ene,2,6-dimethyl-6-[4-methyl-3-pentenyl]-	8.707	204	$C_{15}H_{24}$
j	16	Gama Cadinene	9.228	204	$C_{15}H_{24}$
k	17	9,12,15-octadecatrienoic acid,methyl ester	9.804	292	$C_{19}H_{32}O_2$
	18	Phytol	9.874	296	$C_{20}H_{40}O$
L	19	Linolenic acid	10.160	278	$C_{18}H_{30}O_2$

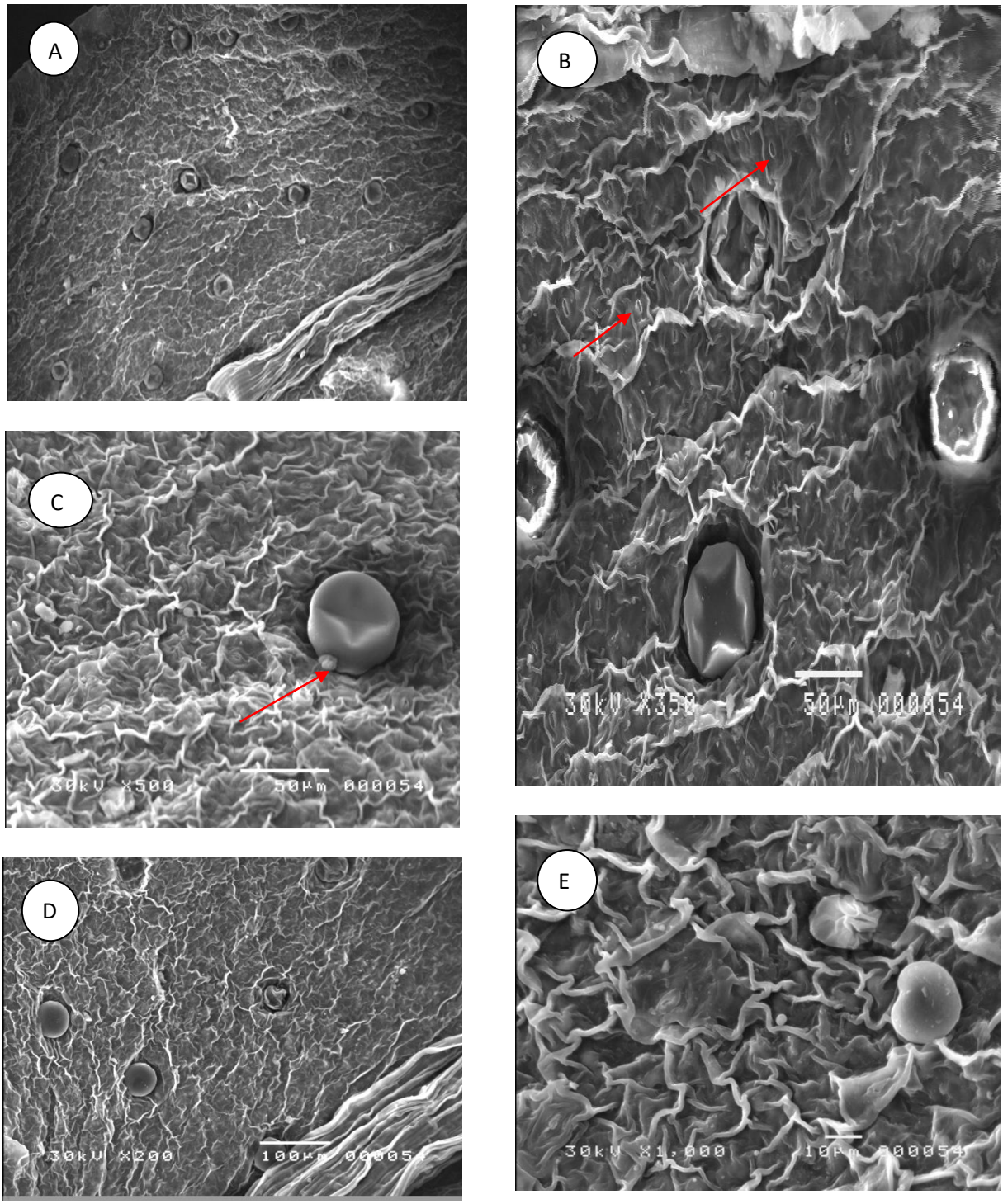

Fig 1. Shows an SEM micrograph of the adaxial leaf surface. A, Showing epidermis covering flat part of nectar glands (arrow); B: cells of secretory epidermis covering inner and outer side of protrusion; C: stomata located at the level other epidermal cells (arrow); D, E: showing presence of pollen grains on epidermal surface.

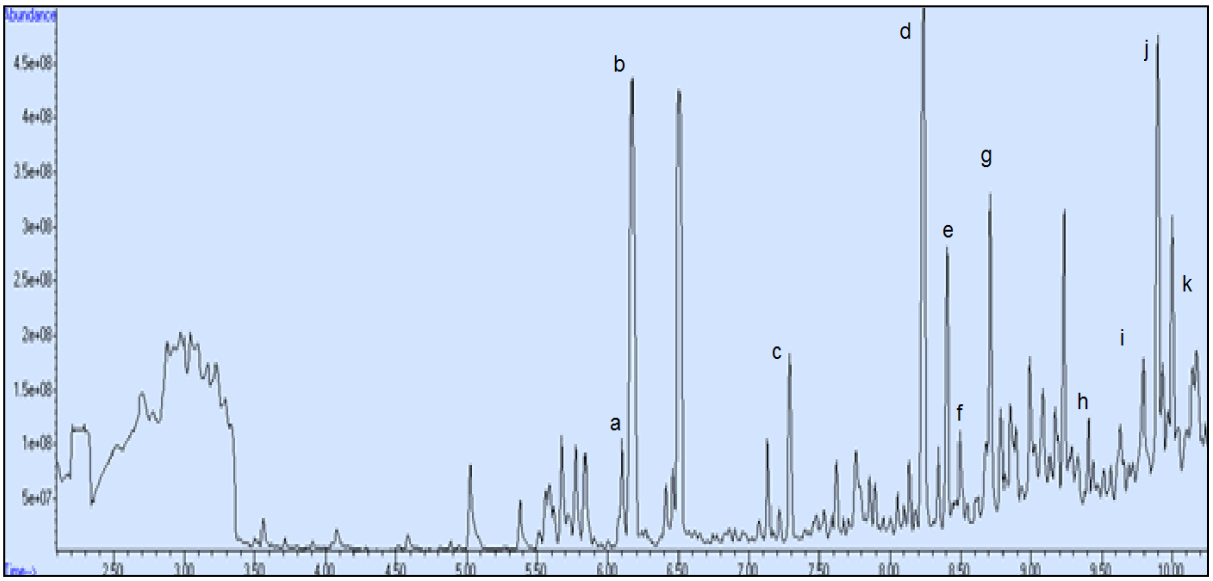

Fig 2. Analysis of leaf extracts of *Ocimumbasilicum* L from the hexane fraction obtained by GC/MSon a stainless steel capillary column 30 m_0.25 mm (1.0 µm film thickness): a, camphene; b, 1,8-Cineol = Eucalyptol; c, L- ,alpha-Terpeniol; d, Eugenol; e, Methyl Eugenol; f, Beta Elemene; g,Pachoulene; h, gamma Cadinene; i,9,12,15-octadecatrienoic acid,methyl ester; j, Delta Cadinene; k, Linolenic acid.

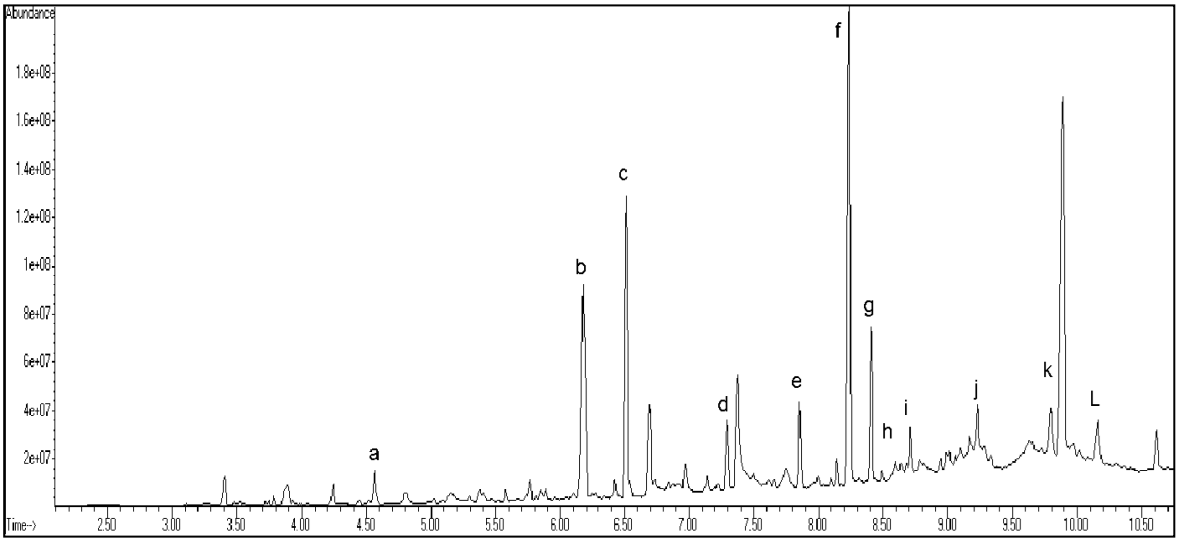

Fig 3. Analysis of leaf extracts of *Ocimumbasilicum* L from the methanol fraction obtained by GC/MS on a stainless steel capillary column 30 m_0.25 mm (1.0 µm film thickness): a, Furfural; b, 1,8-Cineol; c, Beta-Linalool; d, L- ,alpha-Terpeniol; e, Alpha-Fenchyl acetate; f, Eugenol; g, Methyl Eugenol; h, Beta-Elemene; i,Bicycle[3.1.1]hept-2-ene,2,6-dimethyl-6-[4-methyl-3-pentenyl]-; j, Gama Cadinene; k, 9,12,15-octadecatrienoic acid,methyl ester; L, Linolenic acid.

stickiness of the secretion [19]. Most of these diterpenes are phytotoxins and feeding deterrents to a large number of plant feeding mammals, insects and other pathogens, as mentionedin previous studies for many other species secreting terpenoids[20].The results showed that Phenolic compounds which were identified in the hexane fraction, have also been suggested as important defense mechanism against herbivores, insects and pathogens [21].In addition, the sticky lipophilic material was composed mainly of diterpenes, phenols and/or stearic acid. These secondary metabolites probably serve also as important defense against as mentioned above. According to[22], coating of the adaxial leaf surface with these terpenoids, phenols and stearic acid in *Xerophyt aviscosa* may due to minimize loss of water through increased light reflectance, thereby reducing leaf temperature in a desiccation tolerant plant. Figure 4 revealed overlay GC/ MS chromatography for all samples (100, 250, 500, 750 ppm) extracted with n-hexane, showing that for all treatments, nearly all peaks are ideal. These results indicate that Pb until 750 ppm has a slight effect on the chemical composition of sweet basil oils. The same results were observed for methanol samples (Fig. 5).

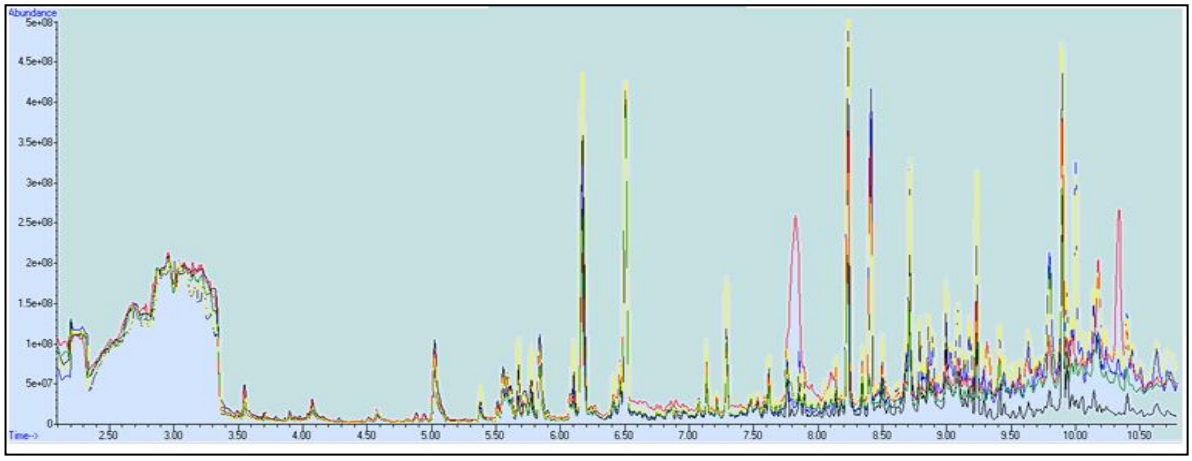

Fig 4. Anoverlay GC/MS chromatograph for "n-Hexane" samples.

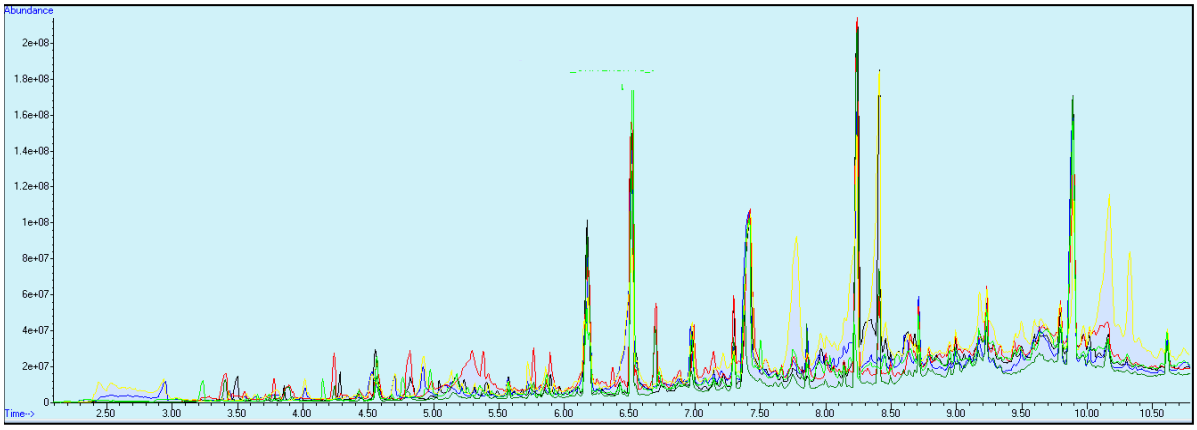

Fig 5. An overlay GC/MS chromatograph for "Methanol" samples.

4 Conclusions

It may be concluded that *Ocimumbasilicum* is tolerant to lead treatments until 750 ppm. The chemical composition of the essential oils of basil is slightly altered. Also secretory tissue of basil was not affected, thus we may recommend sweet basil cultivation in Pb enriched soils of Egypt.

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