



A Survey of The Effect of Some Heavy Metals in Plant on The Composition of the Essential Oils Close to Veshnaveh-Qom Mining Area

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ABSTRACT

The purpose of this study was to evaluate the effect of copper and silver in the plants on the mining area and the ingredients of essential oils. Plants collected from the same family, but they were collected randomly from various regions. The concentration of heavy metal in plant samples collected from Veshnave, Qom, Iran have been determined by Flame Atomic Absorption Spectrometry (FAAS). The chemical composition of essential oil isolated by hydrodistillation from *Tanacetum polycephalum* and was determined using Gas Chromatography/Mass Spectrometry (GC- MS). Results obtained from unidirectional variance analysis suggest that there is a meaningful statistical difference between various regions.

Key words: FAAS; Microwave; Copper; Silver; GC- MS; *Tanacetum polycephalum*.

INTRODUCTION

The plants for growing need relatively low amount of metal ions. Natural soil consists of great amount of metal elements. Despite the selective absorption in root cellular membrane, it is possible a few metal ions are found in plant tissues¹. Heavy metals are important environmental pollutants and they will be toxic as the result of human activities in natural and agricultural areas. The reason of toxicity of heavy metals in higher concentration is that they disturb some vital factors of body. Some metals are found naturally in the body and are essential for

health. Naturally, low-concentration heavy metals are found and they are considered low-quantity metals. They are toxic in high concentration or lead to inefficiency and defect in other low-quantity metals³. In high concentration of metals, substitution with essential metals is occurred and as essential metals play important role in formation of pigments and enzymes, the pigments are disrupted and existing elements in soil will be unsuitable for plant growth and biological variety is eliminated^{13,14}. Heavy metals have no role in the body as a metal and they may be extremely toxic⁴.

Copper has roles in proteins structure, photosynthesis electrons transport, mitochondrial respiration, oxidative tension, cell wall metabolism. Copper (Cu) is a cofactor in enzymes as copper/zinc superoxide, cytochrome-c oxidize, amino oxidize, lactase, plastocyanin, and polyphenol oxidize. Copper is effective in movement of iron in cells¹. The plants need copper as a micronutrient matter for growth. Silver soluble salts namely silver nitrate is toxic in concentration above two grams. Silver compounds could be absorbed easily by body tissues and blue or black pigments are created in the skin. One of the suitable methods to measure metals in various materials is using FAAS technique¹⁵. The present study applied this method to measure heavy metals in plant tissue. Determining low quantities of metals in plants was accomplished by digestion with acid or mixture of acids by microwave. The elements were measured by suitable apparatus techniques⁶. Microwave digestion technique is used for multiple samples and short digestion time^{7,8,9}.

EXPERIMENTAL

Reagent and Standard

All reagents were of analytical grade. Double deionized water was used for all dilution. Nitric acid (HNO₃) with purity 65%, H₂O₂ with purity 30% were of pure quality (Merck, Germany). Cu (1.0–5.0 mg/L) and Ag (0.5–5.0 mg/L) were prepared by dilutions from stock solutions of 100 mg/L.

Collection of plants

Plants used in the research from close, 5 and 10 Km around the Veshnave Mine, Qom, Iran in May 2012. Plants were of family *Corpositae*, a genus *Tanacetum polycephalum*. After the plants were collected, dried and powdered, it was homogeneous¹⁰.

Essential oil extraction

The dried parts from *Tanacetum polycephalum* were subjected to hydrodistillation for 3 hours. The essential oil obtained was separated from water with 0.5 ml hexan normal. To analyze the essence, GC-MS is used.

Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS analysis was performed by Hewlett-Packard equipped with a HP-5MS column

(30m × 0.25 mm, film thickness 0.32 mm). Helium used as the carrier gas (1 ml/min). The oven temperature increased from 60°C to 220°C at a rate of 6°C/min.

Identification of components

Retention indices (RI) were calculated by using retention times of *n*-alkanes (C6–C24) that were injected after the oil at the same temperature and conditions. The compounds were identified by comparing their RI with those reported in the literature, and their mass spectrum was compared with those reported in Wiley Library¹⁷.

Sample digestion and analysis

To digest the plant samples, microwave is used. 7 mL of HNO₃ (65% v/v) and 1 ml hydrogen peroxide (30% v/v) were added to 0.5 g dry weight (DW) of tissue and digestion was performed. The extract was filtered and diluted to 25 ml. The metals (Cu and Ag) in the surface water and the plant extracts were analyzed by FAAS (Varian-spectraAA-240)^{7,11}.

RESULTS AND DISCUSSION

The solutions after digestion are used for analysis of copper and silver. The samples (n=9) were collected in particular, from three different areas. Mean values and standard deviations of Cu and Ag in different areas were tabulated in Table 2.

RSD of method for 9 successive measurement of copper was achieved 0.36 % and for silver as 1.13%. LOD for copper is 0.01% and for silver 0.025% and R for copper is ranging 90.15% to 103.9% and ranging 93% to 106% for silver. According to the chart observed this plant susceptible to absorb Cu more than Ag.

Essence analysis by GC-MS

The chemical composition of each oil was tabulated in table 2.

18 compounds were characterized in the oil in region 1 close to mine with camphor (27.18%), cinnamic aldehyd (11.68%), 1,8-cineole (9.06%) and borneol (8.24%) as main compounds. 16 compounds were characterized in the oil in region 2 near the mine (5km) with camphor (25.07%), borneol (14.81%) and 1,8-cineole (11.89%) as main

Table 1: Mean values and standard deviations of metals in plant

	Region 1	Region 2	Region 3
mg/L copper in dry plant	23.46±0.51	6.72±0.61	5.42±0.55
mg/L silver in dry plant	3.02±0.50	2.08±0.52	1.18±0.52

Each value in this table shows the metal concentration in dry plants. Region 1 is in the mine, region 2 is 5km from the mine and region 3 10km from the mine.

Table 2: The identified compositions in essence of different regions

Compound	KI*	Composition in region 1(%)	Composition in region 2(%)	Composition in region 3(%)
α -pinene	934	3.85	5.54	6.27
Camphene	950	3.51	3.35	2.76
Sabinene	973	1.74	2.76	2.81
β -pinene	978	1.13	1.83	1.86
1.8 cineole (Eucalyptol)	1033	9.06	11.89	12.89
Gamma-pinene	1069	2.41	2.43	2.43
Chrysanthenone	1142	1.74	4.29	2.34
Camphor	1172	27.18	25.07	23.21
Benzenepropanal	1178	4.79	0.00	0.00
Borneol	1190	8.24	14.81	18.39
4 Terpineol	1197	4.18	4.30	4.27
Alpha terpineol	1208	2.99	2.83	1.67
Cinnamic aldehyd	1296	11.68	2.89	0.00
Bornyl acetate	1305	6.32	3.13	11.30
Alpha-terpinyl acetate	1364	4.86	9.92	1.24
Germacrene	1515	1.11	1.63	3.47
Spathulenol	1618	2.38	1.37	2.31
Farnezol	1645	0.37	0.00	3.36

Each value in this table shows compositions in essence in dry plants. *KI: Kovats Index, **region 1, is in the mine, region 2, is 5km from the mine and region 3, 10km from the mine.

Table 3: Identified terpens in various regions essence

	Compound in region 1 (%)	Compound in region 2 (%)	Compound in region 3 (%)
MH	12.64	15.91	16.13
OM	53.39	63.19	63.77
SH	1.11	1.63	2.31
OS	2.75	3.79	6.86

Each value in this table shows terpens in essential oil, * MH: Monoterpene Hydrocarbon; OM: Oxygenated Monoterpene; SH: Sesquiterpene Hydrocarbon; OS: Oxygenated sesquiterpene. **Region 1 is in the mine, region 2 is 5km from the mine and region 3 10km from the mine.

compounds. camphor (23.21%), borneol (18.39%), 1,8-cineole (12.89%) and bornyl acetate (11.30%) were the major compounds in the region 3 from high distance from mine. The identified compounds in this study are two types of monoterpenes and sesquiterpenes. The structure of Monoterpenes and Sesquiterpenes compounds is divided into hydrocarbon and oxygenated compounds (Table 3).

Mean values and standard deviations of Cu and Ag in different areas were tabulated in Table 5. The highest concentration of Cu and Ag was observed near the mine and the lowest value was observed at distances of 10 km from the mine. Our results indicate the significant differences between the mean values of Cu and Ag in the different regions ($F(\text{Cu}) = 3025.3$; $F(\text{Ag}) = 171.3$; $p < 0.001$); alternatively, by increasing distance from the mine, the concentration of Cu and Ag decreases. The

results of Duncan test showed that the average of Cu and Ag concentration near the mining areas was significantly higher than 5 km and 10 km from the mine ($p < 0.05$); moreover, mean Cu and Ag concentration was significantly higher in the 5 km area of mine than 10 Km area.

The values of MH in different areas shown in Table 3. As shown in the charts, by increasing the copper and silver concentration, monoterpene hydrocarbon concentration is increased, too. Mean values and standard deviations of MH in different areas shown in Table 4. The highest concentration of MH was observed at distances of 10 km from the mine and the lowest value was observed near the mine. Our results indicate the significant differences between the mean values of MH in the different regions ($F = 954.2$; $p < 0.001$); on the other hand, by increasing distance from the mine, the concentration of MH would be increased.

Table 4: Comparison of mean concentrations (Zn, MH, OM and SH) in different regions using unidirectional variance analysis and Duncan's test results

	Region 1	Region 2	Region 3	F	P
mg/L copper	23.46±0.51	6.72±0.61	5.42±0.55	3025.3	0.001<
mg/L silver	3.02±0.50	2.08±0.52	1.18±0.52	171.3	0.001<
MH (%)	12.64±0.86	15.91±1.01	16.13±1.12	954.2	0.001<
OM (%)	53.39±0.54	63.19±0.89	63.77±0.85	1662.5	0.001<
SH (%)	1.11±0.78	1.63±0.57	2.31±0.51	237.5	0.001<
OS (%)	2.75±0.75	3.79±0.51	6.86±0.76	529.7	0.001<

*Values "Mean ± SD" is shown; **MH: Monoterpene Hydrocarbon; OM: Oxygenated Monoterpene; SH: Sesquiterpene Hydrocarbon; OS: Oxygenated sesquiterpene; ***region 1 is in the mine, region 2 is 5km from the mine and region 3 10km from the mine.

Table 5: Pearson's correlation coefficients among the studied variables (n=9)

Variable	Cu	Ag	MH	OM	SH	OS
Cu	1					
Ag	0.9020	1				
MH	-0.9999	-0.8983	1			
OM	-0.9998	-0.8954	0.9999	1		
SH	-0.8598	-0.9960	0.8855	0.8521	1	
OS	-0.7406	-0.9581	0.7350	0.7305	0.9798	1

MH: Monoterpene hydrocarbon; OM: Oxygenated monoterpene;

SH: Sesquiterpene hydrocarbon; *Significant at $P < 0.05$, **Significant at $P < 0.01$,

***Significant at $P < 0.001$

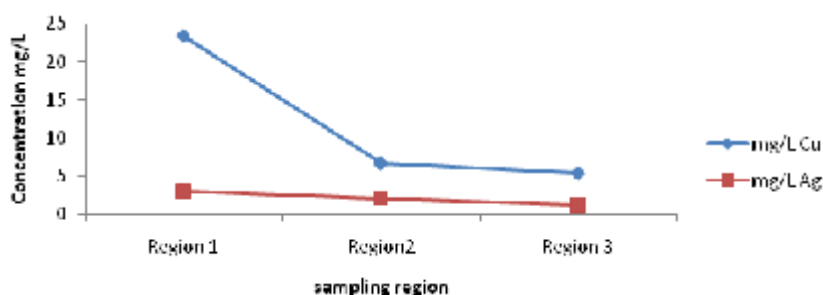


Fig. 1: The investigation of changes of metals concentration with distance from mine.
*Region 1 is in the mine, region 2 is 5km from the mine and region 3 10km from the mine

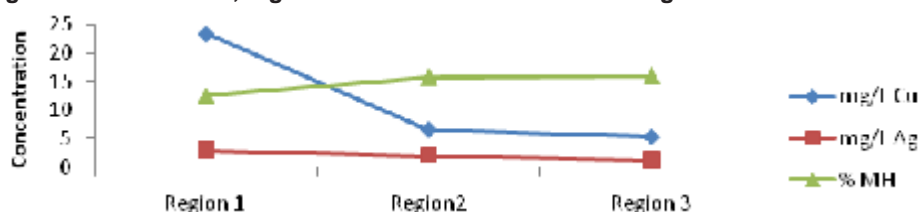


Fig. 2: The study of the effect of distance and copper and silver concentration on monoterpane hydrocarbon concentration

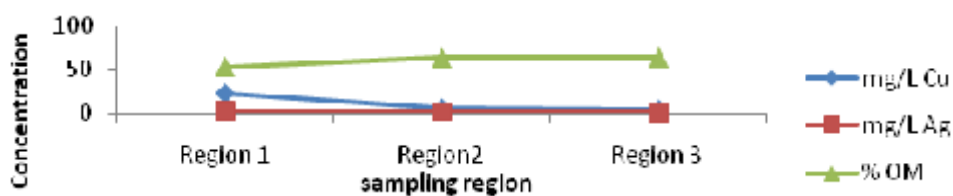


Fig. 3: Evaluation of the effect of distance, copper and silver concentration on concentration of oxygenate monoterpene

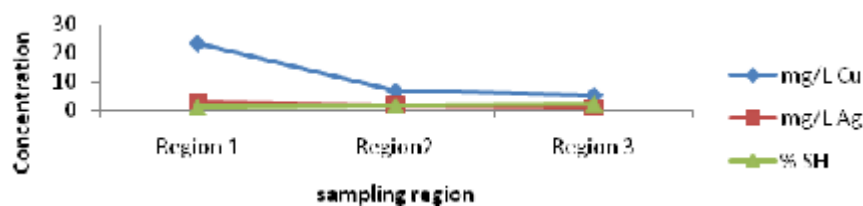


Fig. 4: The evaluation of the effect of distance and copper and silver concentration on concentration of Sesquiterpen hydrocarbon

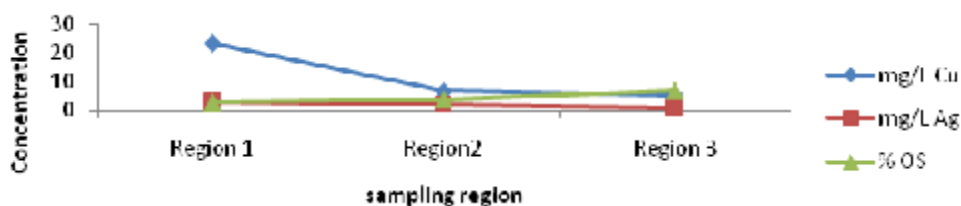


Fig. 5: The evaluation of the effect of distance and copper and silver concentration on concentration of oxygenated sesquiterpene

Duncan test results showed that the average of MH concentration near the mining areas was significantly lower than 5 km and 10 km from the mine ($p < 0.05$).

Comparison of mean concentration of metals on OM in different regions

The values of MO in different areas shown in Table 3. As shown in the charts, as by increasing the copper and silver concentration, oxygenated monoterpenes concentration is increased. Mean values and standard deviations of OM in different areas shown in Table 4. The highest concentration of OM was observed at distances of 10 km from the mine and the lowest value was observed near the mine. Our results indicate the significant differences between the mean values of OM in the different regions ($F = 1662.5$; $p < 0.001$); on the other hand, by increasing distance from the mine, the concentration of OM would be increased. Duncan test results showed that the average of OM concentration near the mining areas was significantly lower than 5 km and 10 km from the mine ($p < 0.05$).

The values of SH in different areas shown in Table 3. As shown in the charts, as by increasing the copper and silver concentration, sesquiterpene hydrocarbons concentration is increased. Mean values and standard deviations of SH in different areas shown in Table 4. The highest concentration of SH was observed at distances of 10 km from the mine and the lowest value was observed near the mine. Our results indicate the significant differences between the mean values of MH in the different regions ($F = 237.5$; $p < 0.001$); on the other hand, by increasing distance from the mine, the concentration of SH would be increased. Duncan test results showed that the average of SH concentration near the mining areas was significantly lower than 5 km and 10 km from the mine ($p < 0.05$).

The values of OS in different areas shown in Table 3. As shown in the charts, as by increasing the copper and silver concentration, oxygenated sesquiterpene concentration is increased.

Mean values and standard deviations of OS in different areas shown in Table 4. The highest concentration of OS was observed at distances of

10 km from the mine and the lowest value was observed near the mine. Our results indicate the significant differences between the mean values of OS in the different regions ($F = 529.7$; $p < 0.001$); on the other hand, by increasing distance from the mine, the concentration of OS would be increased. Duncan test results showed that the average of OS concentration near the mining areas was significantly lower than 5 km and 10 km from the mine ($p < 0.05$).

Pearson's correlation coefficient was used in order to investigate the relationship between studied variables, as tabulated in Table 5. There is a positive correlation between Cu and Ag ($r = 0.9020$). The negative relationship between Cu, Ag and MH ($r_{Cu} = -0.9999$; $r_{Ag} = -0.8983$), OM ($r_{Cu} = -0.9998$; $r_{Ag} = -0.8954$), SH ($r_{Cu} = -0.8598$; $r_{Ag} = -0.9960$) and OS ($r_{Cu} = -0.9999$; $r_{Ag} = -0.8983$) was besides observed.

Copper concentration should be low in plants. Due to the Redox feature of copper, its high concentration is dangerous. The mean content of copper in plant tissue is 10 microgram/gram. Excess copper avoids plant growth and is involved in important cellular processes as photosynthesis and respiration. The plants growing in high copper concentration show reduction in biomass and chlorotic signs. Low chlorophyll content and changes of chloroplast structure and composition of thylakoid membrane in leaf are found under such growth conditions, namely destruction of grana lamellae and stroma lamellae and the increase of size and number of plastoglobuli are observed. Based on the adverse effects of copper and its important role, copper reduction, as in the structure of the identified compound in essence there is carbon-carbon double bond and carbon-oxygen double bond, copper reduces these bonds and changes their structure.

CONCLUSION

This study showed that by increasing the distance from the Veshnave-Qom mine, the concentration of Copper and Silver in *Tanacetum polycephalum* plant decreases. Besides, increase in copper and silver concentration in the above-mentioned plant leads to more increase in terpene

compound, particularly oxygenated monoterpenes and oxygenated sesquiterpenes.

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