

Essential Oil Constituents of *Dracocephalum moldavica* L. Grown under Salt Stress and Different Sources of Soil Amendment

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Abstract: Salt stress is a major a biotic stress in plant agriculture, adversely affects crop productivity and quality in Egypt and other countries that depend on irrigation water for crop growth. The plants differ in their ability to grow under saline conditions. The biosynthesis and metabolism of the essential oil are strongly influenced by environmental factors. This study was conducted to evaluate the essential oil constituents of *Dracocephalum moldavica* grown under salt stress (0, 1500, 3000 and 4500 ppm NaCl) and the importance of using pyrite, gypsum and elemental sulphur as the different sources of soil amendment. The essential oil was characterized by high percentage of oxygenated monoterpenes and the major components were geranial (16.75 – 55.19%), geranyl acetate (9.14 – 33.06%) and neral (12.51 – 22.21%) followed by geraniol (0.31 – 10.99%), than nerol (1.58 – 3.21%). As salinity levels increased the percentage of geranyl acetate decreased and both geranial and neral increased. The principal compound, geranyl acetate was increased with salinity levels increased and treated with pyrite, while geranial recorded the highest relative concentration (42.20 – 55.19%) with plant irrigated with the low level of salinity 1500 ppm NaCl and treated with gypsum and elemental sulphur, respectively. Also 3000 ppm of NaCl mixed with gypsum or elemental sulphur recorded a considerable percentage of geranial (45.20 – 46.14%). The results of this study clearly demonstrated that dragonhead plants can be grown in newly reclaimed soil in Egypt under salt stress and the high alkalinity associated with arid soils can be countered by application of different sources of soil amendment i.e. agricultural sulfur, gypsum and pyrite.

Key words: *Dracocephalum moldavica* • Elemental S • Essential oil • Gypsum • Pyrite • Salt stress

INTRODUCTION

Essential oils and their volatile constituents are used widely to prevent and treat human disease. The possible role and mode of action of these natural products is discussed with regard to the prevention and treatment of cancer, cardiovascular diseases including atherosclerosis and thrombosis, as well as their bioactivity as antibacterial, antiviral, antioxidants and antidiabetic agents [1]. *Dracocephalum moldavica* L. belongs to the family Lamiaceae is an annual plant native in central Asia and naturalized in Eastern and Central Europe [2]. It is used in folk medicine as painkiller and for treatments of kidney complaints, against toothache and colds as well as antirheumatism [3], regulating platelet aggregation of rate [4], stimulated ovulation in female rats and rabbits [5] as

well as it is used as antitumor [6]. It has been used as antioxidant, antiseptic and stimulant [2]. The volatile oil content of *Dracocephalum moldavica* and its composition showed great variation due to plant origin, climate conditions, sowing time and soil fertility [2, 3, 7-11]. The content and composition of secondary metabolites in medicinal and aromatic plants are affected by agricultural practices. Salinity is one of the troublesome factors affecting plant production and agricultural sustainability in many regions of the world as it reduces the value and productivity of the affected land, mainly occurs in arid and semiarid conditions [12] and particularly in newly reclaimed areas in Egypt, where the precipitation is not enough to leach the excess soluble salt from root zone. Salinity problems can also occur in irrigated agriculture particularly when poor quality water

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is used for irrigation [13, 14]. Plants differ in their ability to grow successfully under saline conditions and to accumulated high concentration of salts in their tissues. Increasing the level of the soluble salts in the soil solution tends to increase its osmotic pressure and/or cause an individual ion toxicity [15], which leads to decrease in the water and nutrient uptake by plants. For this reason, several management practices can be adopted in this regard to reduce the adverse effects of the use saline water for irrigation. Sulphur has been recognized to enhance plant growth through its oxidization by soil micro-organisms to sulphuric acid which in turn lowers soil pH, improves soil structure and increases the availability of certain plant nutrients, notably phosphorus and several of micronutrient.

The objective of the present study was to evaluate the essential oil constituents of *Dracocephalum moldavica* grown under salt stress and the importance of using pyrite, gypsum and elemental sulphur as the different sources of soil amendment.

MATERIALS AND METHODS

This study was carried out in the greenhouse of the National Research Centre, Dokki, Giza, Egypt to evaluate the essential oil constituents of *Dracocephalum moldavica* grown under salt stress and the importance of using pyrite, gypsum and elemental S as the different sources of soil amendment. The experiment was laid out in factorial experiment in complete randomized design of all combinations between irrigation with saline water (0, 1500, 3000 and 4500 ppm NaCl) and different sources of sulphur (0, pyrite, gypsum and elemental sulphur S), each treatment was replicated three times. Certified seeds of dragonhead plant were obtained from Saturei-Ka Zahradni Bohnankraut in Poland and propagated under Egyptian conditions. A basal dose of farmyard manure (120 g/pot) was added 15 days before sowing and sulphur was applied at the rate of 150 kg S/feddan (one feddan=0.42

ha) through the application of 3.38 g/pot pyrite (FeS₂), 7.65g/pot gypsum (CaSO₄) and 1.8 g/pot elemental sulphur (S). The seeds were sown on October 15th in earthenware pots (30 cm in diameter) coated with plastic bags then, filled with 12 kg sandy soil. Many holes were made at the bottom of the plastic bag for water drainage. After two weeks from sowing the plants were thinned to one seedling/pot till the end of experiment. The physical and chemical characteristics of the used soil were determined according to Black *et al.* [16] as presented in Table 1.

All treatment received basal fertilizers of ammonium nitrate (33.5% N), calcium superphosphate (15.5% P₂O₅) and potassium sulphate (48% K₂O) at 2 g/pot for each and were added twice, the first after one month from sowing and the second at one month later. All pots were irrigated with tap water up to one month from sowing, after that the irrigation with saline water started using sodium chloride salt (NaCl) at the rate of 1500, 3000 and 4500 ppm, in addition to the control treatment (irrigated with tap water). The plants were collected at full flowering stage on May during the growing season. The essential oil of the fresh herbage was obtained by hydrodistillation in a Clevenger type apparatus for 3h. The collected oils were dehydrated over anhydrous sodium sulfate. The oils were subjected to gas chromatography using a Varian Vista 6000 FID model equipped with a 2-mx, 1/8 daunter, stainless steel, 3% OV-101 column. The flow rate of the carrier gas (nitrogen) was maintained at 50 ml/min and the column temperature was programmed from 50 to 200°C at the rate of 2.5°C/min. The injection port temperature was maintained at 180°C and the detector at 240°C. The relative percent of the oil constituent was determined using a Varian 4270 integrator and oil constituents were identified by matching retention times with those of authentic samples injected with under the same conditions.

Statistical Analysis: Simple correlation coefficients were determined according to Gomez and Gomez [17].

Table 1: Some physical and chemical characteristics of the used soil.

Physical properties						Chemical properties							
						Cation				Anion			
Sand	Silt	Clay	Texture	pH	EC dS/m	K ⁺	Na ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	HCO ₃ ⁻	CO ₃ ⁻	SO ₄ ⁻
87.7	10.0	2.3	Sandy	8.4	0.32	0.13	0.98	1.17	1.10	0.67	1.63	nil	0.90

Table 2: Essential oil constituents of *Dracocephalum moldavica* L. as affected by salt stress

Essential oil constituents	NaCl concentration (ppm)				Mean
	0	1500	3000	4500	
Myrcene	0.27	0.18	0.20	0.17	0.20
Phellandrene	0.49	0.22	0.21	0.21	0.28
Caryophyllene	1.46	1.38	0.93	1.01	1.19
Linalool	2.72	2.56	2.28	2.45	2.50
Neral	17.82	18.45	18.80	18.83	18.47
Geranial	19.13	35.19	34.17	23.91	28.10
Neryl acetate	2.49	1.78	1.47	1.49	1.81
Geranyl acetate	30.36	20.71	18.97	25.23	23.82
Geraniol	9.33	0.65	0.56	0.50	2.76
Nerol	2.91	2.19	1.77	1.65	2.13
Total identified constituents	86.98	83.30	79.36	75.45	81.27
Total hydrocarbon constituents	2.22	1.78	1.34	1.39	1.67
Total oxygenated constituents	84.76	81.52	78.02	74.06	79.60

Table 3: Simple correlation coefficients and regression equations between salinity stress levels and some essential oil constituents of *Dracocephalum moldavica* L.

Constituents	Correlation Coefficient	Regression equation
Total identified constituents	-0.99	$Y = -3.853x + 90.90, R^2 = 0.99$
Total hydrocarbon constituents	-0.93	$Y = -0.293x + 2.415, R^2 = 0.99$
Total oxygenated constituents	-0.99	$Y = -3.56x + 88.49, R^2 = 0.997$
Geranial	+0.22	$Y = 0.00x + 26.10, R^2 = 0.047$
Geranyl acetate	-0.43	$Y = -0.001x + 26.38, R^2 = 0.188$
Neral	+0.93	$Y = -0.00x + 17.96, R^2 = 0.863$

RESULTS AND DISCUSSION

Effect of Salinity Stress: The main constituents of *Dracocephalum moldavica* essential oil as affected by different levels of salinity are shown in Table 2. The GC analysis identified the main compound as geranial in all treatments except at 0 and 4500 ppm and ranged from 19.13 to 35.19% of the total identified constituents. The second main compound was found geranyl acetate for all treatments except 0 and 4500 ppm where it is considered the first one and it ranged from 18.97 to 30.36% of the total identified constituents. Neral was identified as the third main constituent in the essential oil of the all different salinity levels. The mean values of main compounds indicated that, the non significant negative relationship between salinity and geranyl acetate (- 0.43) and can be expressed by the regression equation: $Y = 0.001X + 26.38$ and $R^2 = 0.188$ (Table 3). Non significant positive relationship between salinity and geranial (+ 0.22) and can be expressed by the regression equation: $Y = 0.00X + 26.10$ and $R^2 = 0.047$. On the other hand, the relationship between salinity and neral gave the high significant positive value (+ 0.93) and can be expressed by the regression equation: $Y = 0.00X + 17.96$ and $R^2 = 0.833$. In other words, it can be noticed that, as salinity levels increased the percentage of geranyl acetate decreased and both geranial and neral

increased. The maximum percentage of geranial (35.19%) was obtained as a result of salinity level at 1500 ppm followed by salinity level at 3000 ppm which gave 34.17%. Untreated plants (control) gave the maximum percentage of geranyl acetate (30.36%) followed by salinity level at 4500 ppm which resulted in 25.23% while, the lowest percentage (18.97%) was obtained from plants treated with salinity at 3000 ppm. Salinity level at 4500 ppm gave the highest value of neral (18.83%) against untreated plants which gave the lowest one (17.82%).

The results presented in Table 2 indicated that, the total identified constituents ranged from 75.45% to 86.98%, while the hydrocarbon constituents ranged from 1.34% to 2.22% in the identified compounds. On the Other hand, total oxygenated constituents ranged from 74.06 to 84.76% in the identified compounds. The high significant negative relationship between salinity with total identified constituents (- 0.99), total hydrocarbon constituents (- 0.93) and total oxygenated constituents (- 0.99) and can be expressed by regression equations as presented in Table 3. The minimum percentage of hydrocarbon compounds (1.34%) was observed with plants treated with 3000 ppm, while, the maximum percentage (2.22%) was obtained from untreated plants (control). Moreover, the highest percentage of oxygenated constituents (84.76%) was obtained as a

Table 4: Essential oil constituents of *Dracocephalum moldavica* L. as affected by different sources of soil amendment.

Essential oil constituents	Sources of soil amendment				Mean
	0	Pyrite	Gypsum	Elemental S	
Myrcene	0.23	0.17	0.19	0.26	0.21
Phelandren	0.24	0.26	0.29	0.32	0.28
Caryophyllene	1.10	1.16	1.16	1.32	1.19
Linalool	2.51	2.43	2.30	2.96	2.55
Neral	21.45	18.19	20.84	17.88	19.59
Geranial	22.15	18.82	33.48	35.85	27.58
Neryl acetate	1.66	1.55	1.92	2.19	1.83
Geranyl acetate	25.46	30.66	17.87	21.33	23.83
Geraniol	0.49	2.78	2.64	2.52	2.11
Neral	1.58	2.06	2.20	2.29	2.03
Total identified constituents	76.87	78.06	82.88	86.91	81.18
Total hydrocarbon constituents	1.57	1.58	1.63	1.90	1.67
Total oxygenated constituents	75.30	76.48	81.24	85.01	79.51

Table 5: Simple correlation coefficients and regression equations between different sources of soil amendment and some essential oil constituents of *Dracocephalum moldavica* L.

Constituents	Correlation Coefficient	Regression equation
Total identified constituents	+0.98	$Y=3.494x + 72.44, R^2=0.953$
Total hydrocarbon constituents	+0.86	$Y=0.104x + 1.41, R^2=0.744$
Total oxygenated constituents	+0.98	$Y=3.389x + 71.03, R^2=0.954$
Geranial	+0.86	$Y=5.576x + 13.63, R^2=0.742$
Geranyl acetate	-0.59	$Y=-2.518x + 30.12, R^2=0.348$
Neral	-0.57	$Y=-0.806x + 21.60, R^2=0.327$

result of control against plants salinized with 4500 ppm which gave the minimum value (74.06%). The reduction in essential oil constituents may be due to disturbance in photosynthesis and carbohydrate production under stress condition [18]. Moreover, Aziz and Taalb [19] stated that salinity level at 3000 and 4500 ppm caused a significant reduction in the essential oil percentage as well as essential oil yield of dragonhead plant as compared with control and the reduction increased with increasing salinity level.

High salinity caused a great reduction in relative growth rate and leaf area ratio, decreased osmotic potential and increased turgor potential were accompanied by enhanced N and Cl concentration in the leave and caused alteration of nutrient uptake [20]. Also, Sangwan *et al.* [21] stated that water stress caused induction of PEP carboxylase activity and essential oil biosynthesis. Moreover, Dharmendra *et al.* [22] found that high residual sodium carbonate in irrigation water significantly decreased the herbage yield and essential oil production of *Cymbopogon citrates*. Dadkhah [23] reported that salinity stress imposes additional energy requirements on plant cells less carbon is available for growth and then less essential oil may be synthesized.

Effect of Different Sources of Soil Amendment: GC analysis indicated the presence of 10 main compounds in essential oil of dragonhead in different sources of soil amendment as shown in Table 4. The major compound in the essential oil was found to be geranyl acetate for control and pyrite which recorded 25.46 and 30.66%, respectively. Geranial is considered the first main constituent when plants treated with gypsum and elemental S which recorded 33.48 and 35.85%, respectively. Neral was identified as the third main constituent in all different sources of soil amendment which ranged from 17.88 to 21.45%. The mean values of main constituents indicated that, there was no significant negative relationship between different sources of soil amendment and both geranyl acetate (- 0.59) which can be expressed by the regression equation:

$Y=-2.518X + 30.12$ and $R^2= 0.348$ and neral (- 0.57) which can be expressed by the regression equation: $Y=-0.806X + 21.60$ and $R^2= 0.327$ as shown in Table 5. On the other hand, it can be noticed that there was a significant positive relationship (+ 86) between different sources of soil amendment and geranial which can be expressed by the regression equation:

$$Y=5.576X + 13.63 \text{ and } R^2= 0.742.$$

Table 6: Essential oil constituents (%) of *Dracocephalum moldavica* L. as affected with salt stress and different sources of soil amendment.

Essential oil constituents	0				1500 ppm NaCl				3000 ppm NaCl				4500 ppm NaCl			
	0	Pyrite	Gypsum	Elemental S	0	Pyrite	Gypsum	Elemental S	0	Pyrite	Gypsum	Elemental S	0	Pyrite	Gypsum	Elemental S
Myrcene	0.21	0.22	0.22	0.42	0.19	0.14	0.18	0.2	0.18	0.21	0.21	0.19	0.19	0.11	0.14	0.23
Phelandren	0.45	0.42	0.51	0.59	0.24	0.17	0.2	0.26	0.21	0.23	0.22	0.18	0.19	0.2	0.21	0.24
β-Caryophyllene	1.41	1.4	1.45	1.58	1.21	1.33	1.34	1.62	0.94	0.91	0.9	0.97	1	0.98	0.94	1.1
Linalool	2.4	2.78	2.81	2.88	2.22	2.11	2.1	3.82	2.11	2.2	2.15	2.64	2.51	2.64	2.14	2.51
Neral	20.99	19.95	18.5	11.85	18.24	16.24	21.2	18.11	16.21	16.66	22.21	20.11	12.51	19.91	21.45	21.45
Geranial	20.52	16.75	19.32	19.92	24.18	19.2	42.2	55.19	26.14	19.2	45.2	46.14	26.15	20.14	27.2	22.15
Neryl acetate	2.5	2.05	2.72	2.7	1.25	1.45	1.72	2.71	1.22	1.34	1.62	1.7	1.32	1.35	1.63	1.66
Geranyl acetate	27.11	29.22	32.06	33.06	27.2	32.11	9.14	14.4	24.2	31.14	8.15	12.4	23.2	30.15	22.11	25.46
Geraniol	10.99	9.3	8.38	8.64	0.6	0.64	0.82	0.52	0.5	0.52	0.81	0.41	0.31	0.66	0.55	0.49
Nerol	2.51	2.82	3.21	3.11	2	2.14	2.11	2.51	1.77	1.62	1.75	1.95	1.66	1.64	1.72	1.58

Data presented in Table 4 illustrated that, total identified constituents ranged from 76.87% to 86.91% where elemental S application gave the highest relative value against control (untreated plants) which produced the lowest one. Total hydrocarbon constituents ranged from 1.57% to 1.90% and gave the same trend as shown with total identified constituents. Moreover, the relative values of total oxygenated constituents ranged from 75.30% to 85.01% where elemental S application gave the maximum relative value. The relationship between the effect of different sources of soil amendment on total identified, hydrocarbon and oxygenated constituents are shown in Table 5, where it can be observed that there are high positive relationships. In this respect, Aziz and Taalb [19] showed that the application of different soil amendments i.e. pyrite, gypsum and elemental sulphur as the different sources of sulphur significantly increased the photosynthetic pigments content, the percentage and essential oil yield in leaves of dragonhead plant grown under saline conditions. These effects might be due to sulphur was possibly improve photosynthesis, by increasing leaf area, enhancing CO₂ fixation per unit leaf area and by improving N metabolism [24]. Moreover, the increase in photosynthesis content due to gypsum may be attributed to the fact that gypsum supplied Ca and S, which are essential nutrients for better yield and qualities. Sulphur is required for the synthesis of S-containing amino acids, protein, chlorophyll and oil [25]. Munn and Singh [26] stated that the application of 20kg/ha⁻¹ as ammonium sulphate or elemental S gave a significantly higher oil yield of palmarosa grown under the semiarid tropical conditions as compared to the control (no S application). On the other hand, Amitabha *et al.* [27] found that gypsum was more effective for increasing oil yield of *Mentha spicata* grown in calcareous loamy sand soils than elemental S and ammonium sulphate.

Effect of the Interaction Treatments Between Salinity Stress and Different Sources of Soil Amendment: Data presented in Table 6 showed the influence of salinity and different sources of sulphur on the essential oil constituents of *Dracocephalum moldavica*. The essential oil was characterized by a high percentage of oxygenated monoterpenes and the major components were geranial (16.75–55.19%), geranyl acetate (8.15–33.06%) and neral (11.85–22.21%) followed by geraniol (0.31–10.99%), than nerol (1.58–3.21%). As salinity levels increased the relative percentage of geranyl acetate decreased and both geranial and neral increased, while geranial recorded the highest relative concentration (42.20–55.19%) with plant irrigated with the low level of salinity 1500 ppm NaCl and treated with gypsum and elemental sulphur, respectively. Also 3000 ppm of NaCl mixed with gypsum or elemental S recorded a considerable percentage of geranial (45.20–46.14%). This effects was a companied with decreasing geranyl acetate (9.14–14.4%). Moreover, variable effects ascending or descending were recorded in the content of some components of dragonhead oil as affected with salinity and different sources of sulphur. The volatile oil content and composition have displayed variation due to plant origin and growth location. In Hungary, Halasz-Zelnik *et al.* [7] reported that the essential oil at flowering stage reached 0.74% and citral was the major component of the oil (30–45%). Shuge *et al.* [28] also identified citral as a major oil constituent. In Finland, Holm *et al.* [8] stated that the maximum percentage (0.62%) of oil occurred during flowering and that the oil consisted of 90% oxygenated acyclic monoterpenes (geraniol, geranial, neral, nerol and geranyl acetate). Galambosi *et al.* [10] stated that the essential oil composition of dragonhead is similar in the northern and southern parts of Finland and the major constituents were geranyl acetate (39.5–43.9%), geranial (21.7–18.3%), neral (14.7–13.8%) and geraniol (5.3–5.8%).

Table 7: Simple correlation coefficients and regression equations between interaction treatments (salinity stress levels + different sources of soil amendment) and some essential oil constituents of *Dracocephalum moldavica* L. plant

Constituents	Correlation Coefficient	Regression equation
Total identified constituents	-0.44	$Y = -0.708x + 87.29, R^2 = 0.198$
Total hydrocarbon constituents	-0.76	$Y = -0.062x + 2.201, R^2 = 0.572$
Total oxygenated constituents	-0.43	$Y = -0.652x + 85.03, R^2 = 0.185$
Geranial	+0.24	$Y = 0.598x + 23.04, R^2 = 0.059$
Geranyl acetate	-0.31	$Y = -0.535x + 28.33, R^2 = 0.096$
Neral	0.16	$Y = -0.099x + 17.62, R^2 = 0.024$

Table 8: Simple correlation coefficient between the main constituents of essential oil of *Dracocephalum moldavica* L. plants.

Items	X1	X2	X3	X4	X5	X6
Total identified constituents X1	0.00					
Total hydrocarbon constituents X2	0.63	0.00				
Total oxygenated constituents X3	0.07	0.60	0.00			
Geranial X4	0.34	0.05	0.50	0.00		
Geranyl acetate X5	-0.03	0.13	0.21	-0.91	0.00	
Neral X6	-0.12	-0.40	-0.12	0.17	-0.45	0.00

In Egypt, El-Gengaihi and Wahba [29] noted the volatile oil is composed mainly of acyclic oxygenated monoterpenes, which account for 93% of the oil constituents. Also in Egypt, Aziz and El-Sherbeny [30] observed that the essential oil of *D. moldavica* was characterized by a high percentage of oxygenated monoterpenes (81.84%–96.05%), with the major compounds being geranial (22.82%–55.83%), geranyl acetate (9.75%–31.48%), neral (16.08%–22.02%) and geraniol (0.42%–16.59%). Moreover, Aziz *et al.* [31] stated that the major components of oxygenated compounds in different growth stages were neral, geranial and geranyl acetate which accounted 82.45, 85.49, 88.0 and 87.7% of the oil. Also, Aziz *et al.* [11] stated that the essential oil of the dragonhead plant grown in newly reclaimed land in Egypt was generally characterized by a high percentage of oxygenated compounds and the major constituents under all agricultural sulfur and ammonium sulfate treatments were geraniol (29.11–42.56%), geranial (14.08–30.94%), geranyl acetate (15.08–23.51%) and neral (10.96–15.35%). In contrast, Shatar and Altantsetseg [9] reported that linalool (67.0%) was the major constituent of the essential oil of *D. moldavica* plants cultivated in Mongolia.

Data presented in Table 7 indicated that, there were no significant relationship between the combination treatments and total identified constituents (-0.44), total oxygenated constituents (-0.43), geranial (0.24), geranyl acetate (-0.31) and neral (0.16). Whereas, there was a significant negative relationship between combination treatments and total oxygenated components (-0.76) which can be expressed by the regression equation:

$$Y = -0.062 X + 2.20, R^2 = 0.572.$$

The Correlation Between the Main Essential Oil Constituents:

Data presented in Table 8, clear the correlation between the main constituents of dragonhead essential oil. It can be noticed that most of the relationship were not significant. There was significant positive relationship between total identified constituents and both total hydrocarbon constituents (0.63) and total oxygenated constituents (0.60). On the other hand, there was high significant negative relationship between geranial and geranyl acetate (-0.91). In our study, the correlation between geranial and neral in various combination treatments was $r = -0.45$. Researchers have illustrated that geraniol can be synthesized by geraniol synthesis from geranyl phosphate (GPP) [32], than by a dehydrogenase and/or oxidase [33] be converted to geranial. Geranial and neral are isomer. The results of this study clearly demonstrate that dragonhead plants can be grown on newly reclaimed soil in Egypt under salt stress and the high alkalinity associated with arid soils can be countered by application of different sources of soil amendment i.e. agricultural sulfur, gypsum and pyrite.

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