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BIOLOGICAL RESERVES OF LIQUORICE (*GLYCYRRHIZA GLABRA* L.) IN THE AZERBAIJANI FLORA AND HEAVY METAL ACCUMULATION

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ABSTRACT: *The article deals with distribution specifications of iron, manganese and nickel in organs of Glycyrrhiza glabra L. The innovation in this research include the study of heavy metal (Fe, Mn, and Ni) accumulation and amount in root and shoot system of G.glabra in areas where these elements were abundant or absent, and investigation of accumulation peculiarities in vegetative and generative organs. The amount of the toxic element – nickel – appeared to be in norm in all organs of the plant. The high number of biogenic elements – iron and manganese - is typical for leaves. The amount of the studied elements in root, which is used for medicinal purposes, is very low, which enables its usage.*

KEYWORDS: Glycyrrhiza Glabra L, Heavy Metals, Technogenic Pollution, Biological Accumulation

INTRODUCTION

The rapid technological innovations require new ecological investigation methods for detecting heavy metals and toxic elements in industrial, pharmacological, dietary products and environmental goods. It is very urgent to define the pollution sources and degrees of the atmosphere, water and soil during environmental monitoring. Not only wild medicinal herbs, but also other fruitful plants (food, feed, etc.) are investigated from ecological point of view.

It is also very important to study the natural resources of wild plants and to maintain their proper use. Plants can be divided into three groups: wild medicinal plants, which have very little resources (*Altheae oficinalis, Berberis vulgare, Potentilla erecta, Acorus* etc.); plants with little resources and less information about the resources (*Crataegus* ssp., *Sambucus nigra* etc.). This group also include some species which require much care (*Betula pendula, Rubus caesius* etc.). The third group includes the Red List species, endangered and/or rare species.

Liquorice is one of the exported plants, which is clinically tested and has many perspectives. There are six species of liquorice in the Azerbaijani flora: *Glycyrrhiza uralensis* Fisch ex DC. (= *G. glandulifera* Waldst. et Kit.), *Glycyrrhiza aspera* Pall., *Glycyrrhiza echinata* L., *Glycyrrhiza foetidissima* Tausch., *Glycyrrhiza macedonica* Boiss & Orhanides, and *Glycyrrhiza glabra* L.

Glycyrrhiza glabra L. is more useful for cultivation and usage, which is a perennial herb and is 50 to 200 cm long. Its trunk is bare, straight, and simple, with branches. Its leaves are alike a complex feather with 5 to 20 cm length, bright, strong, longish, and sticky. Its flowers are 12 mm long, and ovary is white pink. Its fruit is longish straight or a bit bent. The root system consists of primary and lateral roots, and go deeper to 8 m; the plant is kept in soil firmly by

its root system. The aboveground trunk develops from the primary root. The plant blossoms in May-June, fruit is mature in September. *Glycyrrhiza glabra* L. is widespread in Middle Asia, Kazakhstan, Caucasus, Northern Caucasus, and south Europe [Flora of Azerbaijan, 1954]. In Azerbaijan, it is widespread in riverbanks, in shallow ravines, and cultivated areas.

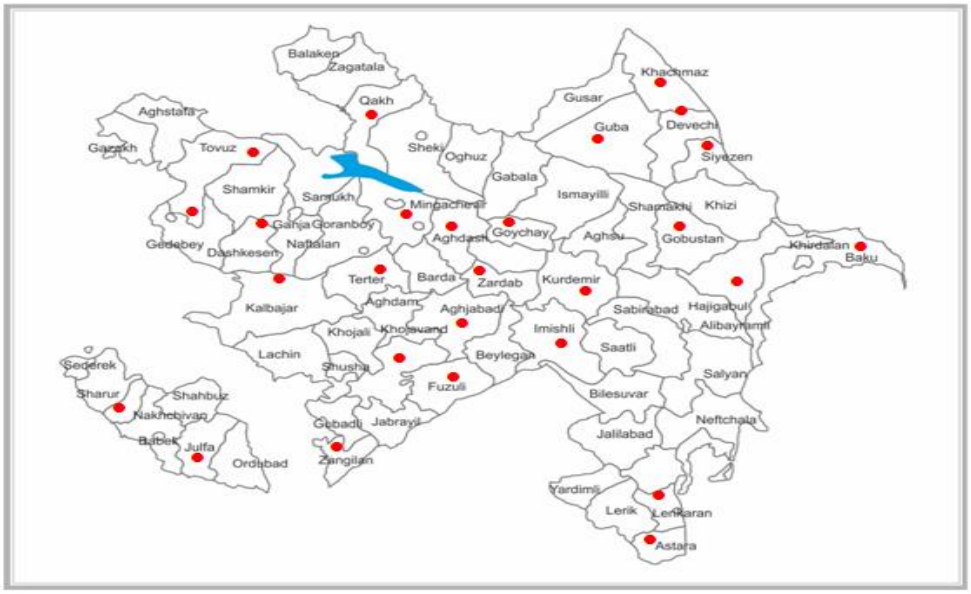
Pharmacological activity is as important, as the natural and active components which define the therapeutic properties of biologically active medicinal plants. *Glycyrrhiza glabra* L. is included in the State Pharmacopeia (DF X, p. 573 and DS 22839-77; 42-0296-2339-02, 42-0273-1781-01 and 0296-2339-02). The colour of the raw material changes from light yellow to brown yellow. It has no smell, tastes sweet, and a bit irritates. All the root parts are considered a high-quality medicinal herb. The glycyrrhizic and glycyrrhetinic acids regulate desoxicorticosterone. It is a cough suppressant, anti-inflammatory, and pain killing remedy. There are medications, such as "Elekasol", "Mirfazin" "Kasmin", and "Roqlidis" against gastrointestinal problems and cough. There is also "Kodelak" in powder form. Dry and semi-solid (0.25 percent ammoniac), made of the root, is considered an elixir for thoracic problems [Mashkovsky, 1977]. The roots contain 23 percent of glycyrrhianic acid, 4 percent of flavonoids, steroids, essential oils, ascorbic acid, bitter substances, pigments, and resin. Sweet liquorice roots contain saponins - glycyrrhazic acid (23 percent, sweeter than sugar), flavonoids (4 percent of likviratine, likviritigenine, isolikviritine), glycyrrhetinic acid, ascorbic acid, steroids, essential oils, sugar, pigment, resin, asparagine, and mucous. The shoot part of the plant consists of tanning agents, flavonoids, essential oils, sugar, and pigment. Roots also contain triterpen saponins, resin, organic acids, and fatty acids [Biologically active substance..., 2001].

The sliced raw materials are packed according the demands of the standards and shelf life is 10 years. Considering all the above-mentioned facts, it is decided to calculate the natural biological deposits of the plant, and define the heavy metals in soils under liquorice.

MATERIAL AND METHOD

The research object was *Glycyrrhiza glabra* L. During the researches not only sources of raw material, but also procurement availability, economical demands in widespread areas (density of population, traffic for transportation, cleanness of the territory etc.) were considered. Therefore, first, the ecological state was studied. The study of wild medicinal herbs and their proper use consist of several stages. First, objectives of the study are studied, which include evaluation of raw material sources, and defining possible annual procurement amount [Krylova et al., 1971].

All the information about the procurement of the raw material in the last five years were gathered, and cartographic materials (1:600 000; 300 000 and 1: 100 000) and map of the areas, where the plant is gathered were prepared (Map 1).



Map 1. *Glycyrrhiza Glabra* L. Distribution Areal

In the next step raw material stock was calculated. Later, in order to study the ecological purity of the plant, heavy metal accumulation in the root and shoot system of the plant was studied. Atomic absorption method is generally used for defining heavy metal accumulation [Alekseev, 2008].

Metal accumulation peculiarities in plant is calculated by biological calculation ratio (BCR) in the following formula:

$$BCR = \frac{\text{element amount in pla}}{\text{element amount in soil}}$$

BCR>10 - the species are the concentration of the studied element.

10 >BCR≥1 - metal is considered a weak accumulation.

1> BCR≥0.1 – keeps the element weaker [Guidelines for the determination of..., 1992].

The data is produced by Microsoft Excel 2003 and Statistics 6.0 software.

EXPERIMENT AND RESULTS

The territory of the Azerbaijan Republic is very complex from geographical point of view. Two lowland stripes – eastern and western – are easily seen in the relief of the studied geobotanical regions of the Lesser Caucasus. Parallel mountain range spreads through in submeridianal direction. The relief here consists of mountain rocks with sediments and lowlands. The northern region of the Lesser Caucasus has a special bio- and geochemical composition. The

territory is characterized by a various poly-metallic spector and anthropogenic disorder. The pollutants here destroy the plants, so some phytomeliorative measures are taken against pollution [Ibadullayeva et al., 2016]. The territory of the Lesser Caucasus is rich in natural resources and iron deposits as well (Dashkesen, Gedebey etc.). Bozgir plateau is one of the studied geobotanical areas. Biogenic metals are accumulated here via river flows. Lowland areas underwent much salinization. But liquorice is a salt-resistant plant, so salt doesn't affect it badly, but vice-versa enriches its composition. The distribution areal of liquorice is humid bank rivers. Mesophytes (*Potentilla reptans* L., *Alopecurus arundinaceus* L., *Glycyrrhisa echinata* L., *G.glabra* L., *Menta arvensis* L., *Agrimonia eupatoria* L. etc.) are typical for these areas.

Biological stock of *Glycyrrhiza glabra* for two years are studied first in selected populations (Bozgir plateau P, Yevlakh P2, Kurdemir P3

Aghdash P 4,Ujar-Goychay P 5-6P Gedebey P7, Gedebey P8, Dashkesen P9, Goygol P10, Bozgir plateau P11) (Table 1).

Table 1. *Glycyrrhiza Glabra* Reserve in Studied Areas (2017-2018)

Regional Selected Population	Distribution Area (h)	Reserve Density (h/t)	Biological Reserve (t)	Used Reserve (t)
2017				
P1	578	8.00	1097	548
P2	350	8.70	453	226.3
P3	636	8.20	1168	584. 9
P4	463	9.40	932.7	466.3
P5	480	17.60	805	402.7
Total:	**2507**	**8.4± 0.05**	**4456.16± 50.84**	**2227.38± 25.42**
2018				
P7	318	8.60	583.00 ± 34.35	291.50 ± 12.10
P8	380	16.60	626	313
P9	138	12.50	490.3	245.1
P10	280	9.76	645	322.5
P11	250	7.70	370	185
Total:	**1366**	**11.03±0.12**	**2714.30± 29.42**	**1357.15± 15.32**

As shows the table the selected populations are satisfactory for procurement.

Roots and stem of the perennial wild *Glycyrrhiza glabra* are gathered in different seasons and used as a medicinal raw material of high quality. The raw material consists of 70-75% of roots, and vegetative organs. The raw material can be re-gathered every year. The researches proved that the root parts left under soil after gathering give new seedlings. The gathered parts are dried in a ventilated area under high temperature.

Heavy metal accumulation in *Glycyrrhiza glabra* has not been studied until now, in the areas stated in the table. Heavy metals are resistant pollutants, however most of them are essential

for living organisms. As a microelement they are engaged in very necessary biochemical processes. Heavy metals exist in soil and plants to some degree. As we know, heavy metals can be included in human diet via food, and trigger different diseases, even malign tumours [Beslanev et al., 2012; Sanitary and epidemiological..., 2011]. When the amount of heavy metals in soil is more than the limit, it causes the pollution of medicinal herbs.

The wide use of *G.glabra* and its ecological purity increases the necessity for the study of specific pollutants of this plant. Selected seno-populations (SP) are studied in general populations of geobotanical regions of the Lesser Caucasus, the Azerbaijan Republic: Bozgir plateau (SP 1), Aghdash (SP 2), Kurdemir (SP 3), Yevlakh (SP 4), Ujar-Goychay (SP 5-6), Gedebey (SP 7, 8), Dashkesen (SP 9, 10), and some area on Bozgir plateau (SP 11). *G.glabra* samples were gathered in eleven seno-populations of average generative state, and soil sections were made in each of them.

After slicing the different organs of the plant, they were dried under necessary condition [State Pharmacopoeia…, 1990]. Each selected soil sample was dried, grinded, and filtered. Iron, manganese, and nickel amount in soil and plant parts – root, leave, trunk, flower, and seeds – were analysed in the laboratory of "Biyan Industrial Park" of the "Biyan Products" LLC.

The technical quality document for liquorice raw material doesn't list the requirements for allowed concentration, so while defining the amount of iron, manganese, and nickel we based on the references [Tamahina et al., 2015].

Iron is essential for leave formation. Iron deficiency weakens photosynthesis and respiration, and causes chlorination. Iron deficiency leads to increase of pH (> 6.0) in very damp soils. The amount of iron for herbs in dry substance is between 50.0 to 240.0 mg/kg; 750.0 mg/kg is considered critical. The allowed amount of iron in soil is considered 3800.0 mg/kg [Beslanev et al., 2012; Perelman, 2000].

The reaction of plant to toxic effects of iron or to its deficiency is different, and it depends on the genotype and species of the plant [Amineva et al., 2010]. Iron concentration in all soils of selected populations doesn't reach the allowed amount, excluding SP 9 (Dashkesen). The lowest concentration was in SP 4 in Aghdash region under river flood areas and near rather washed road soils.

The amount of iron concentration is in norm in the seeds of investigated seno-populations (excluding SP 1-5), but it is over the norm in other organs. The excess amount of iron is in underground parts and trunk. The amount of iron in *G.glabra* organs decreases as follows: underground parts → leaves → flowers → stem (trunk) → seeds.

The underground parts of the plant are considered concentration of iron. In SP 9 the iron amount is maximum in soil and most of iron is accumulated in plant leaves. Because vehicles pollute air while going via soil-covered roads (fig. 1).

Manganese participates in the biosynthesis of chlorophyll and increases its intensity. It has a positive effect on the formation and accumulation of terpenoids, essential oils, steroids and triterpen saponins, glycosides, and alkaloids. And it plays a key role in the management of genetic functions of plants. Manganese is essential for DNA structure of cell and biosynthesis [Alekseev, 2008].

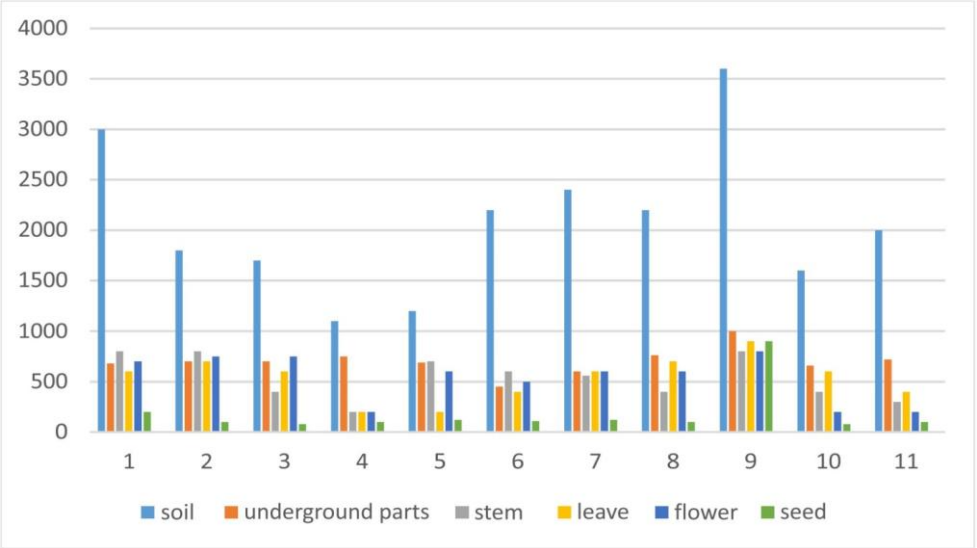


Fig. 1. Iron Amount in *G.glabra* Organs and Soil (mg/kg)

At first, the abundant manganese seems like iron deficiency. If leave cells between veins become yellow, it is called chlorosis. The main difference is that manganese deficiency chlorosis is obvious on upper parts of plant, but iron deficiency is seen on more mature leaves. The amount of manganese in dry substance for herbs is between 25.0 to 250 mg/kg. Over 500mg/kg in dry substance is considered toxic [State Pharmacopeia..., 1990].

The allowed concentration amount of manganese in soils is 600 mg/kg [Reutova et al., 2010]. The amount of manganese in plant organs is not more than the allowed index in the selected seno-populations. The plant has manganese deficiency, the index is between 8 to 50 mg/kg in plant parts. Foliar way is typical for manganese absorption of plant parts. The amount of manganese in *G.glabra* organs is as follows: stem → underground parts → leaves → flowers → seeds (fig. 2).

The chemical elements in soil affect plants, they either increase or decrease each other's effect. The amount of Fe/Mn has an essential index for evaluating plant resistance against iron toxicity: Fe/Mn ratio must not be over 1.5 to 2.5 for normal plant development. Because plant exercises manganese deficiency in rather high indices. This ratio is above norm in all parts of the plant in investigated areas (Table 2).

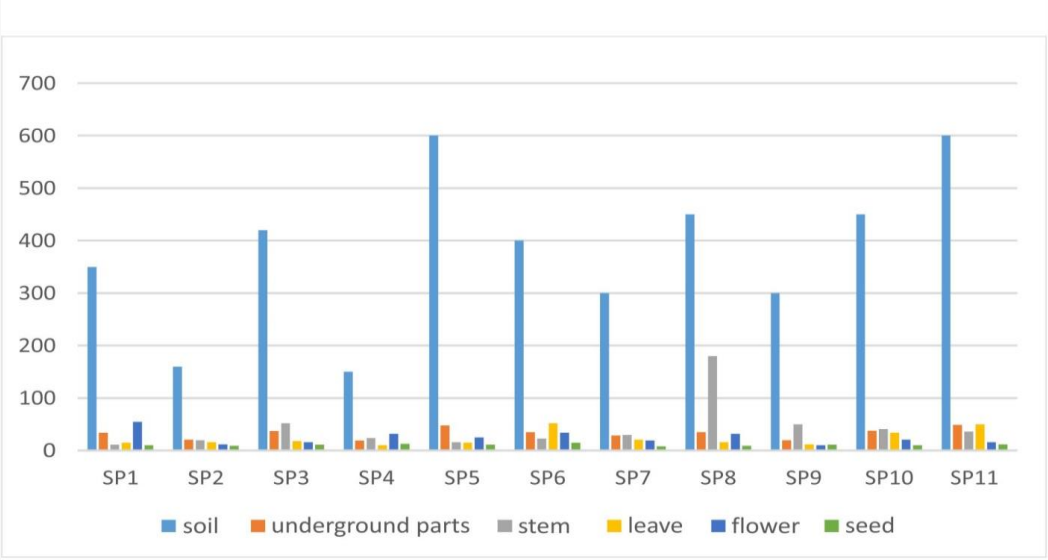


Fig.2. Manganase Amount in *G.glabra* Organs and Soil (mg/kg)

Table 2. Fe/Mn ratio in *G.glabra* L. organs

P	Fe/Mn (mg/kg) in Underground Parts	Fe/Mn (mg/kq) in Stem (trunk)	Fe/Mn (mg/kg) in Leaves	Fe/Mn (mg/kg) in Flowers	Fe/Mn (mg/kg) in Seeds
1	26.8	18.3	10.7	20.0	53.1
2	15.4	7.7	3.4	7.0	6.7
3	12.4	21.9	9.1	21.7	16.3
4	21.9	45.5	59.7	21.0	22.0
5	19.0	33.1	30.6	13.9	7.2
6	17.7	10.4	4.5	28.3	14.3
7	43.4	34.4	15.5	24.9	22.7
8	13.3	26.4	5.5	15.5	8.6
9	26.9	14.9	12.1	18.3	12.1
10	18.0	32.2	9.65	20.8	-
11	13.7	27.0	36.6	22.2	-

The biological activity of nickel takes part in structural formation and activity of DNA, RNA, and proteins, and also in hormonal management of the organism. Excess amount of nickel slows down photosynthesis and transpiration processes, and causes chlorosis of leaves. The toxic effects of the element include decrease of iron enzymes activity, disorder of DNA, RNA, and protein synthesis, and injury of several organs and cells. In Russian soils the allowed concentration amount of nickel is 85 mg/kg [Reutova et al., 2010]. The biological amount of nickel is defined as 85 and 80 mg/kg in plant bodies and soil [State Pharmacopeia..., 1990]. Table 3 describes the research results for seno-populations (fig. 3).

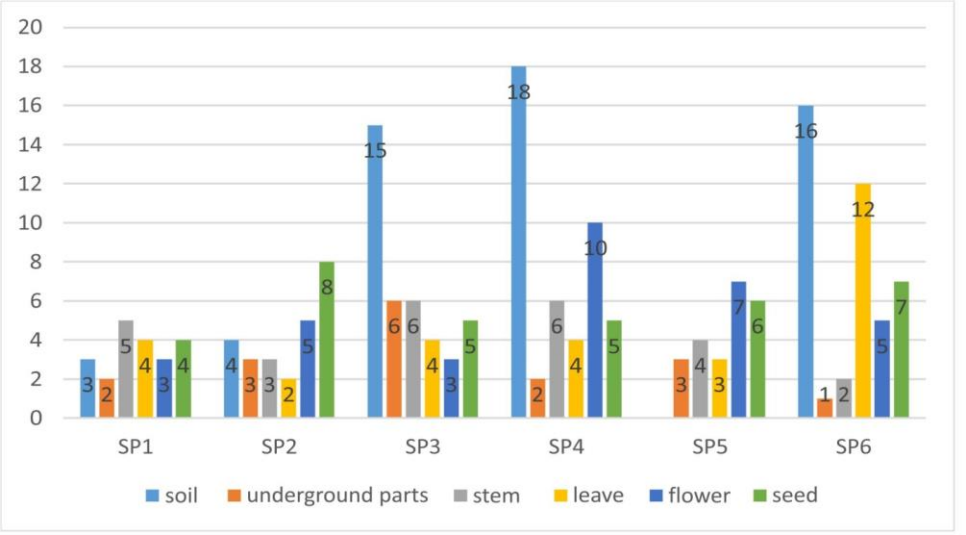


Fig. 3. Nickel Amount in *G.glabra* Organs and Soil (mg/kg)

This research was carried out only in SP 6 in the Lesser Caucasus. The amount of nickel is below the norm in soil and plant organs. Therefore, it has no toxic effect on the plant. The amount of toxic metal – nickel – meets the norms in all parts of the plant. The amount of biogenic metals – iron and manganese – is abundant in the leaves of the plant. The amount of studied elements in the underground parts of the plant, which is used as medicinal product and as a spice in food industry, is in low level. It means the procurement of *G.glabra* as raw material for medicinal purposes and food industry is recommended in the investigated area, which is far from human activities.

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