Influence of ecological factors on pyrrolizidine alkaloids accumulation

A Ya Tamakhina¹, A A Akhkubekova¹, I Sh Dzakhmisheva¹, Z L Kantsalieva¹ and A A Gadieva²

¹Department of Commodity Science, Tourism and Law, Kabardino-Balkarian State Agrarian University, Nalchik, Russia
²Department of Gardening and Forestry, Kabardino-Balkarian State Agrarian University, Nalchik, Russia

E-mail: kbgsha@rambler.ru

Abstract. The article gives results of estimating alkaloids accumulation in the aboveground and underground plant components of family Boraginaceae (Symphytum asperum Lepech., S. caucasicum M. Bieb., Echium vulgare L., Pulmonaria mollis Wulfen ex Hornem.) depending on the ecological conditions of their habitat. Alkaloids content in the aboveground phytomass decreases in the row S. asperum > S. caucasicum > E. vulgare, and in the underground one – S. caucasicum > S. asperum > E. vulgare > P. mollis. At the end of vegetation there is an increase in alkaloids accumulation in roots and decrease in stems and leaves. Rise in air temperature and decrease in precipitation increases alkaloids accumulation in roots and scions. The level of correlation between alkaloids accumulation and microelements content in soil varies from high (Cu, Zn, Mo) to mean (Mn, Pb). The revealed regularities are recommended to discover cenopopulations of S. caucasicum, S. asperum, E. vulgare and P. mollis with maximal accumulation of pyrrolizidine alkaloids or the forecast of their accumulation in certain sections.

1. Introduction
Alkaloids accumulation in plant parts depends on different ecological factors (sunlight intensity, water deficit, high air temperature and relative low moisture, increase of nitrogen and calcium in soil, soil salinification, soil contamination with heavy metals) [1–3]. The greatest alkaloidness is in aboveground parts of the herbaceous plants, it occurs during the blooming period and in rootstocks – during the decline of the aboveground part [4]. When there is a sharp deficit or overplus of mineral elements alkaloid accumulation decreases, which is the base for the universal M-shaped dependence of alkaloids formation and accumulation in plants on their provision with mineral elements [5].

By chemical composition alkaloids of Boraginaceae family refer to the pyrrolizidine group. Toxicity of pyrrolizidine alkaloids is based on their ability to alkylate DNA, cause mutations and oncological diseases [6]. The most toxic pyrrolizidine alkaloids are lasiocarpine, ehimidin and symphytin. The content of pyrrolizidine alkaloids in the plants of Boraginaceae family varies from 0.04 to 0.6 % [7].

Wide spread occurrence of borage family in Russia, including the North Caucasus, application of some parts in medicine necessitates alkaloids content control in different parts of plants. As there is little information on the influence of ecological factors on the pyrrolizidine alkaloids accumulation and their content in some species of Boraginaceae family the purpose of the study was estimation of the level of alkaloid accumulation in aboveground and underground parts of prickly comfrey
Symphytum asperum Lepech.) and Caucasus comfrey (Symphytum caucasicum M. Bieb.), blueweed (Echium vulgare L.) and soft lungwort (Pulmonaria mollis Wulfen ex Hornem.) depending on the climate, orographic and soil factors.

2. Methods and materials
The study was conducted in the Kabardino-Balkarian Republic during 2017–2019. Plant samples (10–12 pieces in every section) were picked up during the blooming and fruit-bearing periods. The collected material was broken up into the rootstocks with roots and stems with leaves. The material was rinsed and dried at the temperature 25–30 °C and crushed.

Alkaloids content was determined by the weight method based on the alkaloids quantitative precipitation with silicotungstic acid solution with the formation of complex salts. The extract was condensed in vacuum at temperature not higher than 30–40 °C, in order not to destroy alkaloids. The analytic repeatability is three-stage.

Soil samples were selected in the area of species habitat from the upper layers of the soil (0–20 cm). The content of heavy metals in active forms (Mn, Cu, Zn, Mo, Pb) in the soil samples has been determined with the atomic absorption method with electro-thermal atomization.

The statistical treatment of the research findings included determination of correlation coefficients (r) and variation coefficients (V).

3. Results
Most habitats of the plants are in steppes (150–450 m below the sea level) and submontane (500–900 m below the sea level) in Kabardino-Balkaria zones. Some parts are limited to the mountain zone – Gill-Su area 2380 m below the sea level), Tashly-Tala village (1120 m below the sea level), lower terrace of the tailing pit of Tyrnyauz tungsten-molybdenum industrial plant (1128 m below the sea level).

Temperature difference in the habitats of P. mollis, S. caucasicum, S. asperum and E. vulgare was 3.37; 2.78; 7.01 and 3.95 °C correspondingly, total precipitation difference was 88.7; 65.3; 88.6; 68.7 mm correspondingly; heights difference was 2116; 593; 860 and 902 m below the sea level correspondingly (Table 1). Significant difference of climatic and orographic conditions allows estimating their influence on alkaloids accumulation.

Soil conditions of the plants habitats also differ. The top soil of the flat part in Kabardino-Balkaria contains liver-colored, meadow, meadow-chernozemic (humusness is 3–4 %), chernozemic (humusness is 5–10 %) and alluvium soils. The main soil types in submontane and mountain parts are grey and reddish forest soils (humusness is 2.8–3.9 %).

Section no. 14 is of special importance among the studied ecotopes. It is a neo landscape of the career-stoker type, a tailing pit terrace of Tyrnyauz tungsten-molybdenum industrial plant. Throughout the period of the enterprise work 80 mln m³ of cleaning rejects wastes have been accumulated containing tungsten, molybdenum and copper-bismuthic concentrates, which are high toxic and have extended solubility in ground waters.

Active forms content of Cu, Mn, Zn, Mo and Pb in sections 1–4 (P. mollis) was 0.22–0.56 (V=46.97 %), 14.15–33.12 (V=35.63 %), 5.52–14.86 (V=41.17 %), 0.11–0.18 (V=30.98 %) and 2.12–4.28 (V=36.5 8%) mg/kg correspondingly.

Active forms content of Cu, Mn, Zn, Mo and Pb in sections 5–9 (S. caucasicum) was 0.34–0.93 (V=42.96 %), 14.32–37.88 (V=40.43 %), 7.46–18.11 (V=32.76 %), 0.08–0.20 (V=38.54 %), 2.32–5.54 (V=41.83 %) mg/kg correspondingly.

Active forms content of Cu, Mn, Zn, Mo and Pb in sections 9–12 (S. asperum) was 0.19–1.46 (V=81.63 %), 14.12–53.26 (V=57.34 %), 5.35–10.34 (V=25.52 %), 0.16–0.20 (V=19.34 %), 1.20–5.53 (V=56.04 %) mg/kg correspondingly.
Table 1. Climatic and orographic conditions of the plants habitat

| Species          | section N | Average annual temperature in March-August, °C | Average annual precipitation total in March-August, mm | Above sea level height, m |
|------------------|-----------|-----------------------------------------------|------------------------------------------------------|---------------------------|
| P. mollis        | 1         | 16.96                                         | 408.8                                                | 847                       |
|                  | 2         | 10.25                                         | 346.7                                                | 2380                      |
|                  | 3         | 16.62                                         | 402.6                                                | 264                       |
|                  | 4         | 16.96                                         | 408.8                                                | 830                       |
|                  | 5         | 16.82                                         | 380.5                                                | 570                       |
|                  | 6         | 18.28                                         | 350.6                                                | 255                       |
| S. caucasicum    | 7         | 19.43                                         | 315.2                                                | 848                       |
|                  | 8         | 19.16                                         | 327.2                                                | 680                       |
|                  | 9         | 18.31                                         | 320.2                                                | 514                       |
| S. asperum       | 10        | 16.37                                         | 408.8                                                | 880                       |
|                  | 11        | 11.30                                         | 364.1                                                | 1120                      |
|                  | 12        | 18.12                                         | 332.3                                                | 260                       |
|                  | 13        | 17.52                                         | 390.8                                                | 226                       |
| E. vulgare       | 14        | 18.38                                         | 328.4                                                | 1128                      |
|                  | 15        | 14.43                                         | 397.1                                                | 620                       |
|                  | 16        | 18.02                                         | 360.4                                                | 648                       |

Active forms content of Cu, Mn, Zn, Mo and Pb in sections 13-16 (E. vulgare) was 0.64–2.48 (V=74.97 %), 22.90–79.34 (V=49.32 %), 5.26–12.52 (V=40.58 %), 0.12–2.76 (V=160.5 %), 2.37–27.67 (V=114.4 %) mg/kg correspondingly.

Soils of P. mollis and S. caucasicum habitat are characterized by mild variability of the active forms of metals, S. asperum – from soft to noticeable high, E. vulgare – from mild to very high. In section 14 the content of Pb and Mo exceeds the sanitary norms (6.0 mg/kg) / the background content (0.2 mg/kg) by 4.6 and 13.8 times correspondingly (fig. 1).

Figure 1. Content of active forms of copper, molybdenum, manganese, zinc and plumbum in the soil of sections (NN 1–16), mg/kg of dry weight

The studied types are characterized by relatively low alkaloids content. The level of alkaloids accumulation in the aboveground phytomass decreases in the row S. asperum > S. caucasicum > E. vulgare > P. mollis, and in the underground – S. caucasicum > S. asperum > E. vulgare > P. mollis (fig. 2).

Alkaloids content in roots of S. caucasicum, S. asperum and P. mollis exceeds this figure in the aboveground part during the blooming and fruit-bearing periods. At the end of vegetation there is an increase of the alkaloids total content in roots (by 21.0–29.2 %) and decrease in stems with leaves (by
7.2–12.8 %). In *E. vulgare* there is a more intensive alkaloid accumulation in the aboveground part. During the fruit-bearing period alkaloids content decreases in stems with leaves by 11.6 %, and in roots increases by 20.0 %. Alkaloids content in the aboveground phytomass of *S. asperum*, *E. vulgare* and *P. mollis* has mild, and in *S. caucasicum* – a noticeable variability. Alkaloid content in roots varies little.

![Figure 2](image)

**Figure 2.** Alkaloids content during the blooming period (a) and fruit-bearing period (b) of plants, % of absolute dry material mass

Alkaloids content in roots of *S. caucasicum*, *S. asperum* and *P. mollis* exceeds this figure in the aboveground part both during the blooming and fruit-bearing periods. At the end of vegetation there is an increase in the total alkaloids content in roots (by 21.0–29.2 %) and decrease in stems with leaves (by 7.2–12.8 %). In *E. vulgare* plants there is more intensive alkaloid accumulation in the aboveground part. During the fruit-bearing period the alkaloid content in stems with leaves decreases by 11.6 %, and in roots increases by 20.0 %. Alkaloid content in the aboveground phytomass in *S. asperum*, *E. vulgare* and *P. mollis* has mild, and in *S. caucasicum* – a noticeable variability. Alkaloids content in roots varies little.

Between alkaloids accumulation in scions, temperature and total precipitation there is average (r=0.53…0.55) and high negative (r=–0.81…–0.83) correlations correspondingly. Correlation between the alkaloid content in the underground phytomass and temperature is weak (r=0.39…0.41), and precipitation is mean negative (r=–0.53…–0.55). On the whole, high air temperature and decrease in precipitation results in alkaloid accumulation increase in roots and scions, that proves the importance of alkaloids in adaptation of plants to climate stress-factors. Temperature and air humidity have more significant influence on alkaloids content in the aboveground parts of plants, than in underground ones, which has been proved by some researches. [1, 4].

Altitude above the sea level correlates weakly with the alkaloids content in scions and roots (r=0.16), which is explained by the position of the most studied ecotopes below the area level with the predominant content of the short-wave radiation in the sunlight [8].

The correlation between alkaloids accumulation in the underground phytomass and metal active forms content in soil decreases in the row Cu>Zn>Mn>Mo>Pb (fig. 3), and in the aboveground – Cu>Zn>Mo=Pb>Mn (fig. 4).
Е. vulgare plants in section N 14 which is contaminated with high content of plumbum and molybdenum have high alkaloids content. Dependence of alkaloids accumulating on the content of Pb and Mo in the soil and phytomass of E. vulgare is proved by high correlation (r=0.86–0.98). This fact proves the effect of heavy metals on the antioxidant support network and the protective role of alkaloids for plants adaptation, growing in conditions of the metal stress [9, 10].

Revealed regularities fit data on the genetic determinacy of bounds between alkaloids and microelements [11], and prove the role of pyrrolizidine alkaloids in plants adaptation to negative ecological factors [4, 12].

The studied plant species differ on alkaloids quantitative composition. Thus, in the aboveground phytomass of S. asperum the main alkaloids are echimidine, sympitine, asperumin, echinatin, heliosupin, acetilechimidin (or its isomers), etyliopsamine (or its isomers), simviridine, and in S. caucasicum – asperumin, echimidine, echinatine, heliotrine, lasiocarpine [13]. In roots of S. asperum echimidine, N-oxides of licopsamine, 7-acetillicopsamine, 3-acetillicopsamine, intermedine, N-oxides of simpitine, 7-acetilsimlandine, simviridine, 7-acetilsimviridine, mioscorpine, triangularine and heloisupine have been identified [14]. In roots of S. caucasicum asperumine, N-oxide of echimidine, echinatine and lasiocarpine have been determined [13]. In the aboveground part of E. vulgare plants 27 alkaloids have been determined, the main was echimidine [15]. There is no data on alkaloids in P. mollis, but in roots and rootstocks of the relative species P. obscura there was intermadine and likopsamine [16].

Most pyrrolizidine alkaloids are highly bioactive substances, but also they have high hepatotoxicity, cancerogenic and mutation effect. There are pyrrolizidine alkaloids, used in medicine. Most important of them are platyfyllin and sarratsyn, which have cholinolytic and relaxing effect and are widely used during spasticity of unstriated muscles of abdominal cavity organs, bronchial asthma, arterial hypertension.

Alkaloids cinoglossin, consolidin and lasiocarpine can cause paralytic of the central nervous system, as they determine partial block of ganglies and disturb impulse coduction to cross-striated muscles. Alkaloid lasiocarpine intoxicates the organism, causes necrosis, fat dystrophy and liver pulp cirrhosis. Symphytine induces liver cancer development. Lasiocarpine and cynoglosin are mutagenic poisons and cause mutagenic malformations. Heliotrin, viridiflorin, heliosupine and laserocarpine are hepatotoxic poisons, and though they have high selective anticancer activity in the experiment they are not used for medical purposes due to high hepatotoxicity and cancinoigecity [17].
In most countries plants which contain pyrrolizidine alkaloids, are forbidden or limited to use. Taking into account that pyrrolizidine alkaloids are a natural component of a range of plants used in medicine and can be used as a part of a food chain, Committee on herbal remedy (CHR, 2014) recommended a maximum permissible daily consumption of pyrrolizidine alkaloids not more than 0.007 mkg/kg of body weight (on average 0.35 mkg/daily for grown-ups and 0.14 mkg/kg for children).

4. Conclusion
The studied plant species of Boraginaceae family accumulate alkaloids weakly (<1%). Alkaloids content in the aboveground phytomass decreases in the row $S. \text{asperum} > S. \text{caucasicum} > E. \text{vulgare}$, and in underground – $S. \text{caucasicum} > S. \text{asperum} > E. \text{vulgare} > P. \text{mollis}$. The level of alkaloids accumulation in rootstocks and roots of $S. \text{caucasicum}$ and $S. \text{asperum}$ exceeds the one in scions; at the end of vegetation there is an increase of total alkaloids content in roots and decrease in stems and leaves. In $E. \text{vulgare}$ plants the alkaloids content is higher in the aboveground part; during the fruit-bearing period alkaidum accumulation decreases in stems with leaves, and increases in roots. Alkaloids content in the aboveground phytomass of the studied species the level of alkaloid accumulation varies little. The relation of alkaloid accumulation in scions and climatic factors is mean or high, and in roots it is weak or mean. The altitude above the sea level has no significant effect on the alkaloids accumulation in the phytomass of the studied plant species. The degree of relation between alkaloids accumulation and microelements content in soil varies from high (Cu, Zn, Mo) to mean (Mn, Pb). The received data evidence the necessity to take into account ecological factors (air temperature, amount of precipitations, soil contamination with heavy metals) when picking plants for medical purposes. The revealed regularities can be recommended for determining cenopopulations of $S. \text{caucasicum}$, $S. \text{asperum}$, $E. \text{vulgare}$ and $P. \text{mollis}$ with maximum content of pyrrolizidine alkaloids and the forecast of their content in certain sections.

References
[1] Babykina A M and Antsupova T P 2012 The influence of some ecology-geographical factors on accumulation of alkaloids in two sorts of poppy Buryat State Univer. Bull. 4 85–87
[2] Terekhin A A and Vandyshhev V V 2008 Cultivation technology of drug plants (Moscow: RUDN)
[3] Rai V, Khatoon S, Bisht S S and Mehrotra S 2005 Effect of cadmium on growth, ultramorphology of leaf and secondary metabolites of Phyllanthus amarus Schum. and Thomm. Chemosphere 61 1644–1650
[4] Roshchina V B and Roshchina V V 2012 The excretory function of higher plants (Saarbrücken: LAP LAMBERT)
[5] Buzuk G N and Lovkova M Y 2011 M-shaped dependance of elements action on alkaloids accumulation in medical plants AgroXXI 7–9 43–45
[6] El-Shazly A and Wink M 2014 Diversity of Pyrrolizidine Alkaloids in the Boraginaceae Structures, Distribution, and Biological Properties Diversity 6 188–282
[7] Stickel F and Seitz H K 2000 The efficacy and safety of comfrey Public Health Nutrition 3 501–508
[8] Kuznetsova G K 2001 Light-physiological study of medical plants”, Genetic resources of medicinal and aromatic plants Int. Conf. dedicated to 50th anniversary of the VILAR botanical garden pp 320–324
[9] Hassanein R A, Hashem H A, El-deep M H and Shouman A 2013 Soil contamination with heavy metals and its effect on growth, yield and physiological responses of vegetable Crop plants (turnip and lettuce) J. of Stress Physiol. & Biochem. 9(4) 145–162
[10] Roos W, Evers S, Hieke M, Tschope M and Schumann B 1998 Shifts of intracellular pH distribution as a part of the signal mechanism leading to the elicitation of benzophenanthridine alkaloids. Phytoalexin biosynthesis in cultured cells of Eschscholzia californica Plant Physiol. 118 349–364
[11] Lovkova M Y, Buzuk G N and Sokolova S M 2008 Genetic aspects of the interrelation between alkaloids and chemical elements in Atropa belladonna L. and Glaucium flavum Grantz plants Appl. Biochem. and microbiol. 44(4) 459–462

[12] Aniszewski T 2007 Alkaloids – secret of life: alkaloids chemistry, biological significance, applications and ecological role (Amsterdam: Elsevier)

[13] Salehi B, Sharopov F, Tumer B et al 2019 Symphytum Species: A Comprehensive Review on Chemical Composition, Food Applications and Phytopharmacology Molecules 24(12) 1–30

[14] Onduso S O, Ng’ang’a M M, Wanjohi W and Hassanali A 2017 Determination of pyrrolizidine alkaloids levels in Symphytum asperum Biofarmasi J. of Natural Product Biochem. 15(2) 65–78

[15] Boppre M, Colegate S M and Edgar J A 2005 Edgar Pyrrolizidine alkaloids of Echium vulgare honey found in pure pollen J. of Agricult. and Food Chem. 53 594–600

[16] Haberer W, Witte L, Hartmann T and Dobler S 2002 Pyrrolizidine alkaloids in Pulmonaria obscura Planta Medica 68 480–482

[17] Zuzuk B M, Kutsik R V, Kostuk I R, Melnichuk G G and Gaiduk R I 2004 Common confrey Symphytum officinale L. (Analytical review) Provisor 19 35–38