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BIOLOGICAL CHARACTERISTICS OF *MELILOTUS OFFICINALIS* (L.) PALL. DEVELOPMENT AND ENVIRONMENTAL SAFETY OF MEDICINAL PLANT RAW MATERIAL

Actuality. *Growing interest in phytotherapy and the use of plant-derived preparations in modern medicine has made it essential to examine the biological development of yellow sweet clover (Melilotus officinalis (L.) Pall.), one of the most widespread medicinal species in the temperate zone. As demand for herbal raw materials rises, so does concern over their environmental safety – particularly the ability of plants to accumulate heavy metals from soil. Insufficient knowledge of M. officinalis ecology and physiology can result in poor yields of high-quality raw material and in the build-up of toxic elements.*

Aim of the study. To characterise the growth and developmental traits of M. officinalis and to quantify the accumulation of heavy metals in its leaf biomass.

Material and methods. *Field trials were conducted on grey podzolic medium-loam soils at the Agronomichne Research Farm (Vinnytsia National Agrarian University, Ukraine). The low-coumarin cultivar Luhansk of M. officinalis was sown. Phenological stages were recorded according to a standard protocol, and plant height was measured on ten permanently marked individuals. Concentrations of Pb, Cd, Cu and Zn in the leaf mass were determined in a certified analytical laboratory. Heavy metal accumulation coefficients were calculated as the ratio of each metal's concentration in plant tissue to its content in the mobile fraction of the soil.*

Research results. *Full emergence of M. officinalis was recorded on day 11, when the sum of active temperatures reached 179 °C and the mean daily air temperature was 17,3 °C. In the sowing year, the crop did not enter budding or flowering stages, following a winter-annual growth pattern and producing aerial biomass up to 130 cm in height. In the second year of vegetation, budding occurred after 87 days and flowering after 93 days, with plants attaining a height of 174 cm. Following biomass harvest, the plants senesced. Heavy-metal analysis revealed that only lead exceeded the maximum permissible concentration by 16,7%. Accumulation coefficients rose with plant age: Pb by 35,3%, Cu by 5,4%, and Zn by 52,8%.*

Conclusion. *M. officinalis exhibits a two-year developmental cycle, achieving peak biomass in the second vegetation year. Under early spring, non-cover sowing, the species behaves as a winter-type crop. The plant selectively accumulates heavy metals, with the highest coefficients observed for Zn and the lowest for Cd and Pb – an aspect that must be considered when using the species as medicinal raw material.*

Key words: *yellow sweet clover, Melilotus officinalis (L.) Pall., medicinal raw material, heavy metals, accumulation coefficient, Pb, Cd, Cu, Zn.*

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БІОЛОГІЧНІ ОСОБЛИВОСТІ РОЗВИТКУ *MELILOTUS OFFICINALIS* (L.) PALL. ТА ЕКОЛОГІЧНА БЕЗПЕКА ЛІКАРСЬКОЇ РОСЛИННОЇ СИРОВИНИ

Актуальність. *Зростання інтересу до фітотерапії та використання рослинних препаратів у сучасній медицині зумовлює необхідність комплексного дослідження біологічних особливостей розвитку буркуну жовтого (Melilotus officinalis (L.) Pall.) – однієї з найбільш поширених лікарських рослин помірного кліматичного поясу. Разом зі зростанням попиту на лікарську рослинну сировину актуальною стає проблема її екологічної безпеки, зокрема, здатність рослин накопичувати важкі* *метали із ґрунту. Відсутність знань про культуру M. officinalis може призвести до отримання замалої кількості якісної лікарської сировини та накопичення токсичних речовин.*

Мета дослідження *– вивчити біологічні особливості росту й розвитку рослин M. officinalis та інтенсивність накопичення в його листовій масі важких металів.*

Матеріал і методи. *Польові дослідження проводили на сірих опідзолених середньосуглинкових ґрунтах Науково-дослідного господарства «Агрономічне» Вінницького національного аграрного університету. Висівали сорт M. officinalis Луганський малокумариновий. Проводили фенологічні спостереження за рослинами згідно з методикою, визначали висоту рослин на десяти постійних рослинах. Вміст важких металів (Pb, Cd, Cu та Zn) у листовій масі визначали в сертифікованій лабораторії. Розраховували коефіцієнт накопичення важких металів як відношення їх умісту в рослинах до вмісту рухомих форм у ґрунті.*

Результати дослідження. *Повні сходи M. officinalis з'явились на 11-ту добу, за накопичення суми активних температур 179 °С і середньодобової температури 17,3 °С. У рік сівби рослини не досягають фаз бутонізації та цвітіння, розвиваючись за озимим типом з інтенсивним надземним ростом до 130 см. На другий рік вегетації фаза бутонізації настає за 87 діб, цвітіння – за 93 доби, з висотою рослин 174 см. Після збирання сировини рослини відмирають. Аналіз важких металів показав перевищення ГДК лише для Pb на 16,7%. Коефіцієнти накопичення зростають з віком рослин: для Pb – на 35,3%, Cu – на 5,4%, Zn – на 52,8%.*

Висновок. *Встановлено дворічний цикл розвитку M. officinalis з максимальною продуктивністю на другий рік вегетації. За безпокривної ранньовесняної сівби культура розвивається за озимим типом. Рослина проявляє здатність до селективного накопичення різних важких металів, що має враховуватися під час використання її як лікарської сировини. Найвищі коефіцієнти накопичення характерні для Zn, найнижчі – для Cd та Pb.*

Ключові слова: *буркун жовтий, Melilotus officinalis (L.) Pall., лікарська сировина, важкі метали, коефіцієнт накопичення, Pb, Cd, Cu, Zn.*

Introduction. Actuality. Yellow sweet clover (*Melilotus officinalis* (L.) Pall.) belongs to the legume family (*Fabaceae*) and is one of the most widespread medicinal plants in the temperate climate zone (Cornara et al., 2016; Doletska et al., 2023). The growing interest in phytotherapy and the use of herbal preparations in modern medicine necessitates a comprehensive study of the biological characteristics of this valuable medicinal species.

The phytochemical profile of *M. officinalis* is distinguished by a high concentration of biologically active compounds, foremost among which are coumarins – specifically coumarin and dicoumarol (Liu et al., 2018). In addition, the plant contains flavonoids (quercetin, kaempferol, rutin), saponins, tannins, essential oils, and ascorbic acid (Ilhan et al., 2020). This diverse ensemble of constituents underpins the broad pharmacological potential of *M. officinalis* (Yıldız et al., 2024).

Therapeutically, *M. officinalis* exhibits pronounced antimicrobial, antioxidant, anticancer, anti-inflammatory, neuroprotective, sedative, spasmolytic, and hypotensive activities (Horváth et al., 2021). Preparations based on this species are traditionally employed to manage chronic venous insufficiency and varicose veins and to enhance microcirculation, owing to the anticoagulant properties of its coumarins (Sun et al., 2020). Contemporary studies further corroborate the efficacy of *M. officinalis* extracts against various cancers (particularly prostate cancer) attributed to newly identified benzoic-acid derivatives (Liu et al., 2018; Parvizpour et al., 2021).

In traditional medicine across various countries, *M. officinalis* is used as an anticonvulsant, analgesic, and expectorant (Ilhan et al., 2020). In modern pharmaceutical industries, dicoumarol – a naturally occurring compound derived from certain plants, including *M. officinalis* – is widely utilized in the production of anticoagulant medications (Ge et al., 2024). Advances in metabolomic research have also identified key bioactive substances, such as oleamide, palmitic acid, and stearic acid, among other valuable metabolites, that significantly influence physiological processes in the human body (Sun et al., 2020; Horváth et al., 2021).

The aerial parts of *M. officinalis* are harvested during the flowering stage for medicinal purposes. The primary raw material consists of the plant's upper shoots (Horváth et al., 2021), although lateral branches may also be cut. The optimal segment for collection includes the top 30 cm of the stems. After cutting, the plant material should be carefully sorted and dried in a well-ventilated area away from direct sunlight or using a mechanical dryer. When using artificial drying methods, stems are typically processed at 40 °C until fully dried. The dried plant mass is then threshed to remove coarse stems. The finished herbal material can be stored for up to two years. It is essential to remove thick stem sections from fresh cuttings, as they contain minimal concentrations of beneficial compounds.

Along with the increasing demand for medicinal plant raw materials, the issue of their environmental safety is becoming increasingly relevant. Of particular concern is the ability of plants to accumulate heavy metals from the soil, which can lead to contamination of the final products (Glavač et al., 2017; Santos et al., 2018; Guo et al., 2023). Research has demonstrated that medicinal plants are capable of accumulating significant amounts of cadmium, zinc, cobalt, manganese, nickel, and lead in concentrations that exceed normal levels by several times (Yao et al., 2019).

The contamination of medicinal plant raw materials with heavy metals is especially pressing in light of the deteriorating environmental conditions and the growing anthropogenic pressure on ecosystems (Asgari Lajayer et al., 2017; Razanov et al., 2020). The accumulation of toxic elements in medicinal plants not only reduces their therapeutic efficacy but also poses a potential threat to human health due to their possible entry into the food chain (Hlihor et al., 2022). Phytoremediation, as an environmentally sound and cost-effective technology, employs plants to remove, degrade, or detoxify hazardous metals (Snitynskyi et al., 2024; Razanov et al., 2024).

Moreover, changes in climatic conditions and anthropogenic impacts on natural ecosystems may affect the biological development characteristics of medicinal plants, including *M. officinalis*, necessitating a detailed study of the plant's adaptive mechanisms and the optimization of its cultivation technologies (Tkachuk et al., 2025). The composition of bioactive compounds in *M. officinalis* can also vary under the influence of fertilizer application, highlighting the need for scientifically grounded approaches to its agricultural production (Doletska et al., 2023).

The growing interest in medicinal plants, particularly *M. officinalis*, has led to an expansion of its cultivated area in agricultural lands. However, a lack of comprehensive knowledge about this crop may result in insufficient yields of leaf-based medicinal raw material and the accumulation of toxic substances. Therefore, a comprehensive study of the biological development characteristics of *M. officinalis* in conjunction with environmental safety concerns is of utmost relevance and significance for the advancement of modern pharmacy and public health protection.

The aim of the study. This study aimed to investigate the biological characteristics of growth and development in *M. officinalis* and to assess the intensity of heavy metal accumulation in its leaf biomass.

Materials and research methods. Field experiments were conducted on grey podzolic medium loamy soils at the Ahronomichne Research Farm of Vinnytsia National Agrarian University. The sown variety was *M. officinalis* Luhanskyi malokumarynovyi (low-coumarin).

Observations focused on the germination and growth features of *M. officinalis* and the prevalence of harmful organisms in its crops. Phenological observations were carried out in accordance with the "Research Methodology" (Razanov et al., 2020). The number of plants entering each growth phase was counted in two consecutive replications of 10 plants each, with the results expressed as a percentage. The onset of a phase was defined as when 10% of plants exhibited changes, and the full phase was marked by 75% of plants reaching it. Visual assessments of *M. officinalis* conditions were performed by monitoring key stages of plant development.

The height of the plants was measured on ten fixed plants using a measuring tape. Air temperature data were provided by the Vinnytsia Regional Hydrometeorological Center. The content of heavy metals (Pb, Cd, Cu, Zn) in the leaf biomass of *M. officinalis* was determined at the certified Scientific Analytical Agrochemical Laboratory of the Educational and Scientific Institute of Agrotechnologies and Environmental Management at Vinnytsia National Agrarian University. The accumulation coefficient of heavy metals in the leaf biomass of *M. officinalis* was calculated as the ratio of the heavy metal content in the plants to the content of mobile forms of heavy metals in the soil.

Research results and their discussion. Germination of *M. officinalis* began on the 7th day after sowing, when the mean daily air temperature was 16 °C and the accumulated growing degree days (GDD) reached 112 °C. Full emergence was recorded on the 11th day, at a cumulative total of 179 °C and a mean daily temperature of 17,3 °C (table 1). At this stage, the plants formed their first simple leaf. The crop stand showed initial infestations of the *Sitona lineatus* Germ. and weed contamination by *Setaria glauca* L.

The first trifoliate leaf appeared on day 21 after sowing, when the accumulated growing degree days (GDD) reached 365 °C. The third trifoliate leaf formed on day 24, at a cumulative total of 421 °C.

Branching began once six leaves had developed, on day 35 after sowing, with an accumulated heat sum of 612 °C. A notable morphological feature is that the lateral branches emerge at right angles to the main stem.

During the sowing year, *M. officinalis* does not reach budding or flowering; instead, it follows a winter-annual growth pattern. The absence of reproductive stages is offset by vigorous aerial biomass production – typical for spring-sown, non-cover crops.

After mowing, regrowth commenced 4–8 days later (depending on soil moisture) when the heat sum reached 166 °C. New shoots arose from buds located on the remaining stubble, but initial growth was slow because the emerging leaves were small and elongation minimal.

Overall, the growth of *M. officinalis* in the first year can be divided into three distinct periods, each characterised by a different rate of height increase (table 2).

In the year of sowing, the growth of *Melilotus officinalis* follows three distinct phases. During the initial stage (0–30 days after sowing), plant height increases slowly, with an average daily increment of about 0,2 cm, reaching a maximum of approximately 5 cm. At this stage, the

Table 1

Progression of growth-and-development phases and accumulated growing degree days (GDD) of *M. officinalis* in the year of sowing

Indicator	Growth and Development Phases									
	Start of emergence	Full emergence	1st trifoliate leaf	3rd trifoliate leaf	Branching	Budding	Onset of flowering	Start of regrowth	Onset of flowering (2nd cut)	Onset of flowering (3rd cut)
Days after sowing to reach phase	7	11	21	24	35	–	–	8	–	–
Accumulated GDD, °C	112	179	365	421	612	–	–	166	–	–

Dash (-) indicates that the phase was not reached during the observation period.

Table 2

Dynamics of *M. officinalis* plant height during the sowing year

	Plant height, cm (days after sowing)												
Days after sowing	20	30	40	50	60	70	80	90	100	110	120	130	140
Plant height, cm	3	5	26	42	95	118	130/*	20	20	27	62	69	69/*

***Note:** An asterisk (*) indicates that mowing of the aerial biomass took place.

Table 3.

Timing of growth and development phases and accumulated growing degree days (GDD) for *M. officinalis* in the second vegetation year

Indicator	Days to reach the growth-and-development phase				
	Start of spring regrowth	Tillering	Branching	Budding	Onset of flowering
Days to reach phase	12,03	37	42	87	93
Accumulated GDD, °C	5,0*	275	343	1 119	1 201

***Note:** 5,0 °C is the mean daily air temperature at the onset of regrowth.

plants are highly vulnerable to weed competition because their above-ground biomass is still poorly developed.

The second phase, marked by intensive growth (30–60 days), shows a sharp rise in daily increments – to roughly 3,0 cm per day, five to six times higher than in the initial period. By day 60, plant height reaches about 95 cm. In the subsequent phase (60–80 days), growth slows: the average daily increment decreases to around 1,2 cm. This deceleration reflects a delay in the transition to generative stages (budding and flowering) which is typical of a winter-annual growth habit under spring, non-cover sowing conditions.

When *M. officinalis* is mown 80 days after sowing at a height of 130 cm, vegetative regrowth begins about 30 days later, initiated by the unfolding of leaves from buds left on the remaining stubble. With a cutting height of 20 cm, four viable buds remain at different levels: the lowest 1 cm above the soil surface, the others positioned 3, 5, and 8 cm higher, respectively.

Regrowth is uneven: plants along plot margins (within ≈ 1 m of the edge) recover more vigorously because of increased light availability. Only 35–40 days after mowing does growth accelerate markedly, and by 60 days plants reach 69 cm. During this second cut, the mean daily height increment is about 1,15 cm. A third cut is not formed. Plants that were not mown in the first cut begin to desiccate in early August (approximately 110 days after sowing) without ever entering the budding or flowering stages.

Spring regrowth of *Melilotus officinalis* typically begins around March 12, when the average daily temperature reaches 5,0 °C (table 3). The plants exhibit high frost resistance: ground-level frosts down to – 6 °C in early April cause no damage, and even frosts of −2 °C in early May do not harm the plants.

Thirty-seven days after the onset of spring regrowth, the formation of shoots (stem elongation) was observed in the plants. This phase occurred on April 18–19, according to the calendar, when the average daily air temperature reached 9,3–10,0 °C and the accumulated active temperatures totaled 275 °C. The branching phase, characterized by the emergence of lateral branches on the central shoot, occurred 42 days after the beginning of spring regrowth (April 23). The bud formation phase was recorded 87 days after regrowth commenced (June 7), and the flowering phase began on the 93rd day (June 13), when the sum of active temperatures reached 1 201 °C.

A noteworthy feature is that the onset of tillering (marking the start of rapid vegetative growth) is governed less by the cumulative heat sum than by the attainment of an average daily air temperature of 9,0–10,0 °C. From the budding stage onward, plant development slows markedly. After the first-cut harvest in the second vegetation year, *M. officinalis* plants senesce and do not regrow, which is a characteristic biological trait of this species.

Because the mean daily temperature at the beginning of spring regrowth was only 5,0 °C and did not rise above 8,0 °C for the following 30–40 days, growth intensity during this period remained low (table 4).

During the first 30 days of the second vegetation year, *M. officinalis* stands grew slowly, with mean daily height increments of 0,20–0,25 cm – slightly higher than in the sowing year, owing to the later onset of spring regrowth.

Once tillering began, growth intensity increased markedly. Average daily increments ranged from 0,6 to 6,2 cm. The highest rates (up to 6,2 cm day^{-1}) were recorded on days 80–90, during the budding stage, while substantial increments of 2,6–3,6 cm day^{-1} were also observed on days 40–50 and 60–80, corresponding to the tillering-branching phases.

In calendar terms, the highest growth increments were recorded throughout May, when the mean daily air temperature averaged 15,0 °C. Plants ultimately reached a maximum height of 174 cm, with mean daily gains of 1,87 cm during the first cut – 25,2% greater than in the first vegetation year (130 cm).

Comparing the growth patterns of the sowing year and the subsequent vegetation year reveals that the initial 30–40 days of each season were similar: average daily height increments ranged from 0,1 to 0,5 cm in both years. Likewise, mean daily gains during the first cut were comparable across the two seasons.

Like many perennial legumes, *M. officinalis* can actively absorb soilborne contaminants owing to its pronounced phytomeliorative and phytoremediation capacities. This ability becomes a liability under highly chemical-intensive cropping systems, where the plant readily accumulates toxicants (especially heavy metals) in its tissues. As a result, the medicinal raw material may become contaminated, undermining both the quality and safety of derived products. Among the various pollutants, heavy metals pose a particular hazard: their excessive accumulation in plant material is linked to disturbances in human physiological functions.

The analysis of heavy metal content in the leaf biomass of *M. officinalis* revealed that only lead (Pb) exceeded the maximum permissible concentration (MPC) by 16,7%, with a measured value of 0,60 mg/kg compared to the allowable limit of 0,50 mg/kg. The levels of other metals were below the MPC: cadmium (Cd) by 40,0% (0,06 mg/kg vs. 0,10 mg/kg), copper (Cu) by 53,0% (4,7 mg/kg vs. 10,0 mg/kg), and zinc (Zn) by 66,0% (17,0 mg/kg vs. 50,0 mg/kg) (table 5).

To evaluate the plant's capacity for heavy-metal uptake, accumulation coefficients (AC) were calculated – the lower the AC, the less the metal migrates from soil to plant tissue. These coefficients rose as the plants aged (table 6), showing that *M. officinalis* displays an enhanced ability to accumulate heavy metals in its second year of vegetation.

In the first vegetation year, the accumulation coefficients were 0,11 for Pb, 0,10 for Cd, 0,70 for Cu, and

Table 4

Dynamics of *M. officinalis* plant height during the second vegetation year

Days after the onset of spring regrowth	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**
Plant height, cm	4	5	8	14	40	50	76	112	174/*

* **Note:** biomass was mown at this stage.

Table 5

Content of heavy metals in the leaf biomass of *M. officinalis* (air-dry basis), M ± m

Heavy metal	**Actual content, mg/kg**	**MPC*, mg/kg**	**Exceedance/Below MPC (%)**
Pb	0,60 ± 0,04	0,50	+16,7
Cd	0,06 ± 0,01	0,10	−40,0
Cu	4,7 ± 0,1	10,0	−53,0
Zn	17,0 ± 0,4	50,0	−66,0

**MPC – Maximum Permissible Concentration according to national health standards.*

Table 6

Accumulation coefficient of heavy metals in the leaf biomass of *M. officinalis* depending on the year of vegetation, M ± m

Vegetation year	Pb	Cd	Cu	Zn
First year	0,11 ± 0,01	0,10 ± 0,01	0,70 ± 0,01	1,87 ± 0,01
Second year	0,17 ± 0,01	0,10 ± 0,01	0,74 ± 0,01	3,96 ± 0,03

1,87 for Zn. In the second year, the coefficients increased by 35,3% for Pb (to 0,17), 5,4% for Cu (to 0,74), and 52,8% for Zn (to 3,96), while the Cd coefficient remained unchanged at 0,10. Zn exhibited the highest accumulation coefficients, indicating a pronounced ability of *M. officinalis* to concentrate this element – particularly in the second vegetation year. Cd and Pb showed the lowest coefficients, which is favourable from the standpoint of the ecological safety of the medicinal raw material.

The results of our study confirm the complexity of the developmental cycle of *Melilotus officinalis* and reveal key patterns that are of practical importance for cultivating this Fabaceae medicinal plant (Cornara et al., 2016). Notably, we found that under early spring, non-cover sowing, the crop follows a winter-annual growth habit – a valuable agronomic trait that has not been described in detail for the Forest-Steppe zone of Ukraine.

The critical threshold for the onset of *M. officinalis* vegetation was a mean daily temperature of 5,0 °C, underscoring the species' pronounced cold tolerance and its adaptation to the climate of the temperate zone. Peak growth rates (up to 6,2 cm per day during the budding stage) indicate an intensive mobilization of the plant's energy reserves as it transitions to the generative phase, a pattern typical of monocarpic developmental strategies.

The detected exceedance of the maximum permissible concentration (MPC) for Pb by 16,7 % is cause for concern, given the widespread use of *M. officinalis* in phytotherapy. Comparable findings were reported by (Santos et al., 2018), who documented Cd accumulation in *Phyllanthus niruri*, and by (Razanov et al., 2020), who observed intensive uptake of heavy metals by *Silybum marianum* L. under crop-rotation conditions – evidence that underscores a broader trend toward heavy-metal bioaccumulation in medicinal plants.

The age-related rise in accumulation coefficients (particularly the 52,8% increase for Zn) aligns with the observations of (Glavač et al., 2017), who reported a greater propensity of perennial medicinal plants to accumulate heavy metals compared with annual species. This phenomenon can be attributed to their more developed root systems and the longer time span available for absorbing elements from the soil.

The selective accumulation of different metals (highest coefficients for Zn and lowest for Cd and Pb) may be linked to the plant's physiological requirements and its metal-transport mechanisms (Hlihor et al., 2022). These findings are critical for the pharmaceutical sector, because *M. officinalis* is widely used to produce anticoagulant preparations owing to its dicoumarol content (Sun et al., 2020). Any exceedance of the MPC for Pb could negate the plant's therapeutic benefits and pose health risks to patients.

The phytoremediation properties identified in *M. officinalis* are consistent with the findings of (Razanov et al., 2024), who documented the capacity of perennial legumes to remove heavy metals from soils. This points to the potential of the species for use in ecological rehabilitation programs on contaminated sites.

The growth and developmental traits identified for *M. officinalis* provide a basis for optimizing its cultivation technology. Because the crop does not enter generative stages during the first vegetation year, a two-year production cycle should be planned, with the main biomass harvest scheduled for the second year. This recommendation aligns with the guidelines proposed by (Doletska et al., 2023) for improving sweet-clover cultivation practices.

Given the rise in heavy-metal accumulation coefficients during the second vegetation year, rigorous soil-quality monitoring is essential, along with agronomic measures that limit the uptake of toxic elements.

Conclusions. Based on the results of the conducted study, specific features of the development of yellow sweet clover *(Melilotus officinalis* (L.) Pall.) and its ability to accumulate heavy metals have been established. Complete seedling emergence of *M. officinalis* occurs on the 11th day after sowing, at a cumulative active temperature of 179 °C and an average daily temperature of 17,3 °C. Branching begins after the formation of six leaves. In the year of sowing, *M. officinalis* does not reach the budding and flowering stages, which is compensated by intensive above-ground vegetative growth up to 130 cm. Thus, under spring no-cover sowing, *M. officinalis* exhibits a winter-type development. In the second year of vegetation, the budding phase begins 87 days after the onset of regrowth, and flowering starts after 93 days, with plants reaching a height of 174 cm. After harvesting the medicinal biomass during the first cut in the second year, *M. officinalis* plants die off and do not regenerate. The accumulation coefficients for heavy metals in the first vegetation year were: Pb – 0,11;

Cd – 0,10; Cu – 0,70; Zn – 1,87. In the second year, these coefficients increased by 35,3% for Pb, 5,4% for Cu, and 52,8% for Zn, while the Cd coefficient remained unchanged. These findings confirm that *M. officinalis* follows a two-year growth cycle, achieving peak productivity in its second vegetation year, and demonstrate the plant's selective accumulation of different heavy metals – an important consideration when using it as medicinal raw material.

Prospects for further research. The findings obtained broaden our understanding of *M. officinalis* biology but require further verification under diverse soil-climate conditions. A key avenue for future research is to examine how different agronomic practices affect the quality of the medicinal raw material and to develop strategies that minimize heavy-metal accumulation without lowering the levels of bioactive compounds.

BIBLIOGAPHY

Asgari Lajayer B., Ghorbanpour M., Nikabadi S. Heavy metals in contaminated environment: destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. *Ecotoxicol Environ Saf.* 2017. № 145. P. 377–390. DOI: 10.1016/j.ecoenv.2017.07.035.

Cornara L., Xiao J., Burlando B. Therapeutic potential of temperate forage legumes: a review. *Crit Rev Food Sci Nutr.* 2016. 56. Suppl 1. P. S149 – S161. DOI: 10.1080/10408398.2015.1038378.

Doletska A., Boretska I., Romanyuk O., Shevchyk-Kostiuk L., Dzhura N. Assessment of the efficiency of technologies for optimizing the cultivation of *Melilotus officinalis* (L.) Pall. on oil-contaminated soil. *Ecological Sciences.* 2023. № 5 (50). P. 15–19. DOI: 10.32846/2306-9716/2023.eco.5-50.2.

Ge W., Zhang X., Wang Q., Mao J., Jia P., Cai J. Dicoumarol attenuates NLRP3 inflammasome activation to inhibit inflammation and fibrosis in knee osteoarthritis. *Mol Med Rep.* 2024. № 29 (6). P. 100. DOI: 10.3892/mmr.2024.13224.

Glavač N. K., Djogo S., Ražić S., Kreft S., Veber M. Accumulation of heavy metals from soil in medicinal plants. *Arh Hig Rada Toksikol.* 2017. № 68 (3). P. 236–244. DOI: 10.1515/aiht-2017-68-2990.

Guo C., Lv L., Liu Y., Ji M., Zang E., Liu Q., Zhang M., Li M. Applied analytical methods for detecting heavy metals in medicinal plants. *Crit Rev Anal Chem.* 2023. № 53 (2). P. 339–359. DOI: 10.1080/10408347.2021.1953371.

Hlihor R. M., Roșca M., Hagiu-Zaleschi L., Simion I. M., Daraban G. M., Stoleru V. Medicinal plant growth in heavy-metal-contaminated soils: responses to metal stress and induced risks to human health. *Toxics.* 2022. № 10 (9). P. 499. DOI: 10.3390/toxics10090499.

Horváth G., Csikós E., Andres E. V., Bencsik T., Takátsy A., Gulyás-Fekete G., Turcsi E., Deli J., Szőke É., Kemény Á., Payrits M., Szente L., Kocsis M., Molnár P., Helyes Z. Analyzing the carotenoid composition of melilot (*Melilotus officinalis* (L.) Pall.) extracts and the effects of isolated (all-E)-lutein-5,6-epoxide on primary sensory neurons and macrophages. *Molecules.* 2021. № 26 (2). P. 503. DOI: 10.3390/molecules26020503.

Ilhan M., Ali Z., Khan I. A., Taştan H., Küpeli Akkol E. The regression of endometriosis with glycosylated flavonoids isolated from *Melilotus officinalis* (L.) Pall. in an endometriosis rat model. *Taiwan J Obstet Gynecol.* 2020. № 59 (2). P. 211–219. DOI: 10.1016/j.tjog.2020.01.008.

Liu Y. T., Gong P. H., Xiao F. Q., Shao S., Zhao D. Q., Yan M. M., Yang X. W. Chemical constituents and antioxidant, anti-inflammatory and anti-tumor activities of *Melilotus officinalis* (Linn.) Pall. *Molecules.* 2018. № 23 (2). P. 271. DOI: 10.3390/molecules23020271.

Parvizpour S., Masoudi-Sobhanzadeh Y., Pourseif M. M., Barzegari A., Razmara J., Omidi Y. Pharmacoinformatics-based phytochemical screening for anticancer impacts of yellow sweet clover, *Melilotus officinalis* (Linn.) Pall. *Comput Biol Med.* 2021. № 138. P. 104921. DOI: 10.1016/j.compbiomed.2021.104921.

Razanov S., Tkachuk O., Lebedieva N., Shkatula Yu., Polishchuk M., Melnyk M., Krektun B., Razanova A. Phytoremediation of heavy metal contamination by perennial legumes. *International Journal of Environmental Studies.* 2024. № 81 (1). P. 216–222. DOI: 10.1080/00207233.2023.2296764.

Razanov S. F., Tkachuk O. P., Razanova A. M., Bakhmat M. I., Bakhmat O. M. Intensity of heavy metal accumulation in plants of *Silybum marianum* L. under field-rotation conditions. *Ukrainian Journal of Ecology.* 2020. № 10 (2). P. 131–136. URL: https://www.ujecology.com/articles/intensity-of-heavy-metal-accumulation-in-plants-of-silybum-marianum-l-in-conditions-of-field-rotation.pdf.

Santos V. S. V., Arantes K. M., Gonçalves E. L., Campos C. F., de Campos Júnior E. O., de Oliveira A. M. M., Pereira B. B. Contamination of soil and the medicinal plant *Phyllanthus niruri* Linn. with cadmium in ceramic industrial areas. *Environ Monit Assess.* 2018. № 190 (5). P. 303. DOI: 10.1007/s10661-018-6693-4.

Snitynskyi V., Razanov S., Hnativ P., Bakhmat O., Kutsenko M., Kolisnyk O. Phytoremediation of 137Cs-contaminated sod-podzolic soil in Northern Polissia by white sweet clover (*Melilotus albus*). *International Journal of Environmental Studies.* 2024. № 81 (1). P. 223–229. DOI: 10.1080/00207233.2023.2270305.

Sun C., Zhao W., Wang X., Sun Y., Chen X. A pharmacological review of dicoumarol: an old natural anticoagulant agent. *Pharmacol Res.* 2020. № 160. P. 105193. DOI: 10.1016/j.phrs.2020.105193.

Tkachuk O., Postoienko V., Bakhmat O., Verheles P., Razanova A., Lysak H., Razanov O., Verhelis V. Biological characteristics of medicinal goatsrue (*Galega officinalis* L.) development and chemical composition of raw material at different sowing periods in the conditions of the Right-bank Forest-steppe. *Fitoterapiia. Chasopys – Phytotherapy. Journal.* 2025. № 1. P. 160–168. DOI: 10.32782/2522-9680-2025-1-160.

Yao J. J., Kong D. D., Luo J. Y., Qin W. J., Qin X. M., Fan Z. W., Yang M. H. Safety evaluation of heavy-metal-contaminated *Astragalus membranaceus* using a health risk assessment model (in Chinese). *Zhongguo Zhong Yao Za Zhi.* 2019. № 44 (14). P. 3094–3099. DOI: 10.19540/j.cnki.cjcmm.20190517.201.

Yıldız I., Başar Y., Erenler R., Alma M. H., Çalımlı M. H. A phytochemical content analysis and antioxidant activity evaluation using a novel method on *Melilotus officinalis* flower. *South African Journal of Botany.* 2024. № 174. P. 686–693. DOI: 10.1016/j.sajb.2024.09.060.

REFERENCES

Asgari Lajayer, B., Ghorbanpour, M., & Nikabadi, S. (2017). Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. *Ecotoxicol Environ Saf.* 145, 377–390. DOI: 10.1016/j.ecoenv.2017.07.035.

Cornara, L., Xiao, J., & Burlando, B. (2016). Therapeutic potential of temperate forage legumes: A review. *Crit Rev Food Sci Nutr.* 56 (Suppl 1), S149 – S161. DOI: 10.1080/10408398.2015.1038378.

Doletska, A., Boretska, I., Romanyuk, O., Shevchyk-Kostiuk, L., & Dzhura, N. (2023). Assessment of the efficiency of technologies for optimizing the cultivation of *Melilotus officinalis* (L.) Pall. on oil-contaminated soil. *Ecological Sciences.* 5 (50), 15–19. DOI: 10.32846/2306-9716/2023.eco.5-50.2 [in Ukrainian].

Ge, W., Zhang, X., Wang, Q., Mao, J., Jia, P., & Cai, J. (2024). Dicoumarol attenuates NLRP3 inflammasome activation to inhibit inflammation and fibrosis in knee osteoarthritis. *Mol Med Rep.* 29 (6), 100. DOI: 10.3892/mmr.2024.13224.

Glavač, N. K., Djogo, S., Ražić, S., Kreft, S., & Veber, M. (2017). Accumulation of heavy metals from soil in medicinal plants. *Arh Hig Rada Toksikol.* 68 (3), 236–244. DOI: 10.1515/aiht-2017-68-2990.

Guo, C., Lv, L., Liu, Y., Ji, M., Zang, E., Liu, Q., Zhang, M., & Li, M. (2023). Applied analytical methods for detecting heavy metals in medicinal plants. *Crit Rev Anal Chem.* 53 (2), 339–359. DOI: 10.1080/10408347.2021.1953371.

Hlihor, R.M., Roșca, M., Hagiu-Zaleschi, L., Simion, I.M., Daraban, G.M., & Stoleru, V. (2022). Medicinal plant growth in heavy-metal-contaminated soils: Responses to metal stress and induced risks to human health. *Toxics.* 10 (9), 499. DOI: 10.3390/toxics10090499.

Horváth, G., Csikós, E., Andres, E.V., Bencsik, T., Takátsy, A., Gulyás-Fekete, G., Turcsi, E., Deli, J., Szőke, É., Kemény, Á., Payrits, M., Szente, L., Kocsis, M., Molnár, P., & Helyes, Z. (2021). Analyzing the carotenoid composition of melilot (*Melilotus officinalis* (L.) Pall.) extracts and the effects of isolated (all-E)-lutein-5,6-epoxide on primary sensory neurons and macrophages. *Molecules.* 26 (2), 503. DOI: 10.3390/molecules26020503.

Ilhan, M., Ali, Z., Khan, I.A., Taştan, H., & Küpeli Akkol, E. (2020). The regression of endometriosis with glycosylated flavonoids isolated from *Melilotus officinalis* (L.) Pall. in an endometriosis rat model. *Taiwan J Obstet Gynecol.* 59 (2), 211–219. DOI: 10.1016/j.tjog.2020.01.008.

Liu, Y.T., Gong, P.H., Xiao, F.Q., Shao, S., Zhao, D.Q., Yan, M.M., Yang, X.W. (2018). Chemical constituents and antioxidant, anti-inflammatory and anti-tumor activities of *Melilotus officinalis* (Linn.) Pall. *Molecules.* 23 (2), 271. DOI: 10.3390/molecules23020271.

Parvizpour, S., Masoudi-Sobhanzadeh, Y., Pourseif, M.M., Barzegari, A., Razmara, J., Omidi, Y. (2021). Pharmacoinformatics-based phytochemical screening for anticancer impacts of yellow sweet clover, *Melilotus officinalis* (Linn.) Pall. *Comput Biol Med.* 138, 104921. DOI: 10.1016/j.compbiomed.2021.104921.

Razanov, S., Tkachuk, O., Lebedieva, N., Shkatula, Yu., Polishchuk, M., Melnyk, M., Krektun, B., Razanova, A. (2024). Phytoremediation of heavy metal contamination by perennial legumes. *International Journal of Environmental Studies.* 81 (1), 216–222. DOI: 10.1080/00207233.2023.2296764.

Razanov, S.F., Tkachuk, O.P., Razanova, A.M., Bakhmat, M.I., Bakhmat, O.M. (2020). Intensity of heavy metal accumulation in plants of *Silybum marianum* L. under field-rotation conditions. *Ukrainian Journal of Ecology.* 10 (2), 131–136. Retrieved from: https://www.ujecology.com/articles/intensity-of-heavy-metal-accumulation-in-plants-of-silybum-marianum-l-in-conditions-of-field-rotation.pdf.

Santos, V.S.V., Arantes, K.M., Gonçalves, E.L., Campos, C.F., de Campos Júnior, E.O., de Oliveira, A.M.M., Pereira, B.B. (2018). Contamination of soil and the medicinal plant *Phyllanthus niruri* Linn. with cadmium in ceramic industrial areas. *Environ Monit Assess.* 190 (5), 303. DOI: 10.1007/s10661-018-6693-4.

Snitynskyi, V., Razanov, S., Hnativ, P., Bakhmat, O., Kutsenko, M., Kolisnyk, O. (2024). Phytoremediation of 137Cs-contaminated sod-podzolic soil in Northern Polissia by white sweet clover (*Melilotus albus*). *International Journal of Environmental Studies.* 81 (1), 223–229. DOI: 10.1080/00207233.2023.2270305.

Sun, C., Zhao, W., Wang, X., Sun, Y., Chen, X. (2020). A pharmacological review of dicoumarol: An old natural anticoagulant agent. *Pharmacol Res.* 160, 105193. DOI: 10.1016/j.phrs.2020.105193.

Tkachuk, O., Postoienko, V., Bakhmat, O., Verheles, P., Razanova, A., Lysak, H., Razanov, O., Verhelis, V. (2025). Biological characteristics of medicinal goatsrue (*Galega officinalis* L.) development and chemical composition of raw material at different sowing periods in the conditions of the Right-bank Forest-steppe. *Fitoterapiia. Chasopys – Phytotherapy. Journal.* 1, 160–168. DOI: 10.32782/2522-9680-2025-1-160 (in Ukrainian).

Yao, J.J., Kong, D.D., Luo, J.Y., Qin, W.J., Qin, X.M., Fan, Z.W., Yang, M.H. (2019). Safety evaluation of heavy-metal-contaminated *Astragalus membranaceus* using a health risk assessment model (in Chinese). *Zhongguo Zhong Yao Za Zhi.* 44 (14), 3094–3099. DOI: 10.19540/j.cnki.cjcmm.20190517.201.

Yıldız, I., Başar, Y., Erenler, R., Alma, M.H., Çalımlı, M.H. (2024). A phytochemical content analysis and antioxidant activity evaluation using a novel method on *Melilotus officinalis* flower. *South African Journal of Botany.* 174, 686–693. DOI: 10.1016/j.sajb.2024.09.060.

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