Biology

Research on the genotoxicity of environmental pollutants on marigold (Tagetes erecta L.) in an urban ecosystem

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Abstract

Purpose of the study – research of fertility of pollen grains of upright marigold (Tagetes erecta L.) in different areas of Almaty.

Hypothesis:
Ecosystems in Almaty are subject to uniform anthropogenic stress.

Stages of research:

1. Collection of marigolds in open gathering places.
2. Preparation of marigold pollen preparations.
3. Counting the number of pollen grains in dusty marigolds to determine fertile and sterile grains.
4. Statistical processing of the results using the student's t-test.
5. Determination of metal content in the soil of marigold collection sites.
6. Analysis and comparison with literature data.
7. Finalizing the work.

Research method:
Pollen test, atomic absorption spectrometry, statistical analysis using the student's t-test.

Results and conclusions:
The results of statistical processing were compared with literature data. In general, at collection sites where a low level of contamination was expected (and a low level of sterile pollen), the amount of sterile pollen was the highest. In places where a high level of contamination was expected, the number of sterile pollen was the least. The results of atomic absorption spectrometry show that pollen sterility is connected with the content of heavy metals in the soil, because at collection sites with a high proportion of sterile pollen, high content of heavy metals such as iron, copper, cobalt, nickel, and chromium was noted.

Areas of the practical application of the data:

The obtained data can be used in biomonitoring of ecosystems in the city of Almaty, as well as in the analysis of pollution of the atmosphere and soil of the city. In addition, the fetched data can be used in a comparative study between the ecosystem of the city of Almaty and other cities with similar environmental problems to identify common signs of various environmental disasters.

Introduction

Increase in human activity leads to higher pollution levels in environment, and the effect of different pollutants (atmospheric, aqueous, etc.) is becoming more severe in big citiesAtmospheric pollutants cause the greatest influence in big cities. Atmosphere, water and soil may contain dangerous pollutants - heavy metals. Heavy metals are dangerous for all types of organisms, causing toxic, mutagenic and carcinogenic effects. In cities, the proportion of heavy metals in the atmosphere is bigger than in wild nature, which means that their influence is also greater. In order to protect human health, it is important to know what amount of heavy metals is present in the ecosystem.

Research on influence of heavy metals on development and growth of plants might be helpful in studying genotoxicity of heavy metals and evaluating threats to human health. Researchers in different countries studied the effect of heavy metals on ability of plants to reproduce. However, in Kazakhstan this topic did not receive enough attention, despite that metallurgical and mining industry are major industries in Kazakhstan. Therefore, an analysis of impact of heavy metals on fertility of living organisms is important for people who live in Kazakhstan. Moreover, studying genotoxicity of heavy metals is essential for maintaining biodiversity in Kazakhstan.

The aim of this research was investigation of pollen grain fertility of marigold (*Tagetes erecta L.*) that grows in conditions of different anthropogenic effect in Almaty city.

Therefore, the following objectives were set:

- identify the level of pollen fertility and infertility of marigold;
- Identify the presence of heavy metals in a soil of gathering points of marigold.

These objectives were achieved with pollen test and atomic-absorption spectrometry.

Literature review

Genetic toxicology

Genetic toxicology is the applied science that aims to study a risk of mutations in somatic and generative cells due to influence of various agents (considering their absorption, dosage, metabolism, food chains, genotype) in order to minimize that risk to minimal for certain individual and population. (1) Genetic toxicology studies influence of pollutants not just on plants, but also on animals. Pollutants can cause carcinogenic and mutagenic effect in living organisms. (1)

Genotoxicity of heavy metals

The “heavy metals” term does not have a certain definition. (2) They could be metals with density higher than that of iron, (3) metals with relatively large atomic mass (4) or metals that cause toxic, carcinogenic and mutagenic effect. This study implies that heavy metals are metals with an atomic mass higher than that of vanadium, i.e., more than 50.9415 u (unified atomic mass units).

Heavy metals, despite their toxicity, in moderate quantities play an important role in life of plants. Iron and manganese are used in redox systems (electron transport chain in photosynthesis) and enzyme activation. Zinc is important for membrane integrity, anaerobic root respiration, protein synthesis and gene regulation and expression. Copper participates in photosynthetic electron transport, respiration and detoxification of superoxide radicals (ions of oxygen with unpaired electrons that can form during cell respiration). (5)

Nevertheless, as it has been mentioned, heavy metals also cause genotoxic effect. Genotoxicity of heavy metals was researched with test-systems of different types. All Previous studies show that excessive amounts of heavy metals negatively affects plants. The Micronucleus test, which shows the number of micronuclei formation after exposure to pollutant (6) in plants (*Tradescantia* hybrid plants, *Vicia faba* and *Allium cepa*), when exposed to lead, cadmium and arsenic produced a significantly high number of micronuclei formation.The results showed that in test-systems made of *Crepis capillaris*, tradescantia and soy silver and chromium are strong mutagens at low concentrations, as toxic as lead and molybdenum. (7)

Heavy metals in atmosphere can cause anomalies in pollen grains of *Pinus sylvestris*, especially nickel, chromium, magnesium and iron. (8) Moreover, increase in quantity of heavy metals in the ecosystem leads to decline of growth in height and diameter of *P. sylvestris*’s trunk, as well as causing disturbances in meristem of seedlings. (9) Analysis of *Chenopodium botrys*, which grew in the environment contaminated with iron and zinc showed disturbances in development of anthers and pollen that influences plant fertility. (10) Analysis of *Reseda lutea*, that grew in heavily contaminated environment showed that excessively large quantities of lead and iron causes disturbances in structure, development of pollen and microspores of *R. lutea*. (11) Growing *Nicotiana tabacum* in polluted environment showed that copper, nickel and mercury cause toxic effect on seed germination of *N. tabacum*, while mercury, cadmium and nickel adversely affect pollen tube length, hindering normal germination. (12)

Heavy metals adversely affect human health. Chromium causes carcinogenic and mutagenic effect, especially in workers of chromium manufacturing industry. (14)

Cadmium accumulates in the liver and kidneys, causing toxic effect on renal and skeletal systems, which results in bone damage.

Lead and its compounds cause toxic influence on neural, renal, hematopoietic and skeletal systems. Lead blocks voltage-dependent calcium membrane channels in neurons, replaces calcium in calcium-sodium ATP pump, interferes with neurotransmitter functioning. (14)

Almaty as urban ecosystem

According to the Department of Statistics of the city of Almaty, by 1st of February, 1,806,833 people live in the city. The city is one of the largest cities in Kazakhstan. From this we can say that the number of vehicles in the city is large, as well as the pollution of the atmosphere.

The level of air pollution in the city is very high, especially the amount of sulfur dioxide and carbon dioxide. Also, the amount of suspended particles (dust) is high. (15)

Pollen in polluted urban areas can contain not only lead and arsenic, but also calcium and iron. Moreover, air pollution increases the amount of pollen allergens, thereby increasing the frequency of allergies in the city. (16) Therefore, the study of the genotoxicity of possible pollutants, including heavy metals, is important for the city.

Materials and Methods

Objects of investigation

Marigold (*Tagetes erecta L.*) was chosen as a research object.This plant is one of the most common in the ecosystem of Almaty city (see Figure 1).

Figure 1. Marigold (Tagetes erecta L.)

Plants were collected in August 2018 in various areas of Almaty city (see Figure 2):

- Collection place №1 – Park of the First President of the Republic of Kazakhstan. The collection place is located far from road.
- Collection place №2 – The intersection of al-Farabi Avenue and Kozybaev Street. Place is situated near a busy highway.
- Collection place №3 – Samal-1 micro district, Zholdasbekov Street. Road is placed near the collection point.
- Collection place №4 – The intersection of Satpayev Street and Baitursynov Street. The collection place is located near the road.
- Collection place №5 – territory of campus of Kazakh National University of al-Farabi. The place is far from the road.
- Collection place №6 – territory of Central Park of Culture and Leisure. The collection place is situated far from the road.

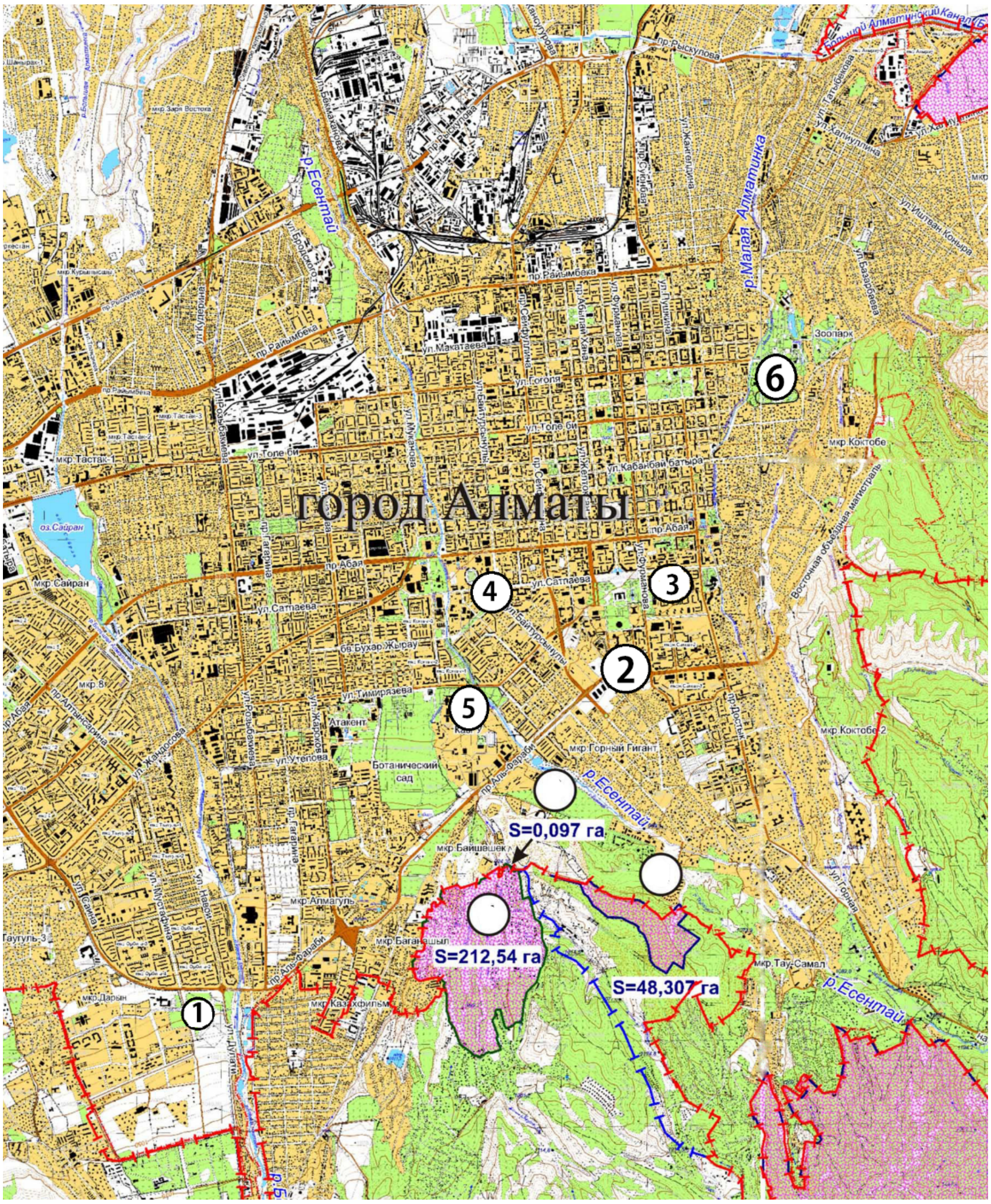

Figure 2. Map of the Almaty city.

7-10 flowers of marigold were collected from each point. In addition, 100-200 grams of soil under the flowers were collected.

Methods

After harvesting, anthers were removed from each flower and placed in Carnoy's fixative (3 parts of ethyl alcohol (96%) for 1 part of glacial aceticacid).

An individual preparation of mature pollen grains was made for each plant. The anthers of a single flower were transferred from a fixative onto a glass slide with adding a drop of 45% acetic acid; the pollen was pulled from anthers with dissecting needles. After removal of empty shells, a drop of dye was added and mixed with a needle, after which the mixture was covered with cover glass.

Acetocarmine was used to stain pollen grains. In order to prepare acetocarmine, 1-2 g of carmine was dissolved in 45 ml of glacial acetic acid and 55 ml of distilled water. Dissolution was carried out in a flask with a reflux condenser on a water bath and heating for 3 hours. After cooling, the dark red solution of carmine was filtered and placed in a pot with a lapped lid.

The analysis of the preparations was performed using a light microscope with magnification of 100x. The amount of sterile pollen was determined for each plant. The total amount of pollen grains at each sampling point was at least 50.

The soil was analyzed using an atomic absorption spectrometer for the presence of heavy metals.

Statistical analysis

To calculate the average error, the following formula (1) was used:

$$S_p = \sqrt{\frac{p(100-p)}{n}}$$

Where:

Sp – Statistical error

p – Fertility (%)

n – Total number of pollen grains

The reliability of the difference (t) between the two points was determined by student's criterion using formula (2):

$$t = \frac{p_1 - p_2}{\sqrt{(Sp_1)^2 + (Sp_2)^2}}$$

Where:

p1 and p2 – % of fertile grains at two points

Sp – Statistical error

Differences were considered significant at $p < 0.05$.

Research results and discussion

The results of the study of sterility of pollen grains of marigold.

Studying sterility level of marigold's pollen grains by using pollen test allows to monitor environmental pollution and obtain information about the degree of genetic stability of the ecosystem.

Fertilized pollen grains are colored with acetocarmine in bright red, dark red or pink color, while sterile grains remain colorless or light beige (see Figure 3-4).

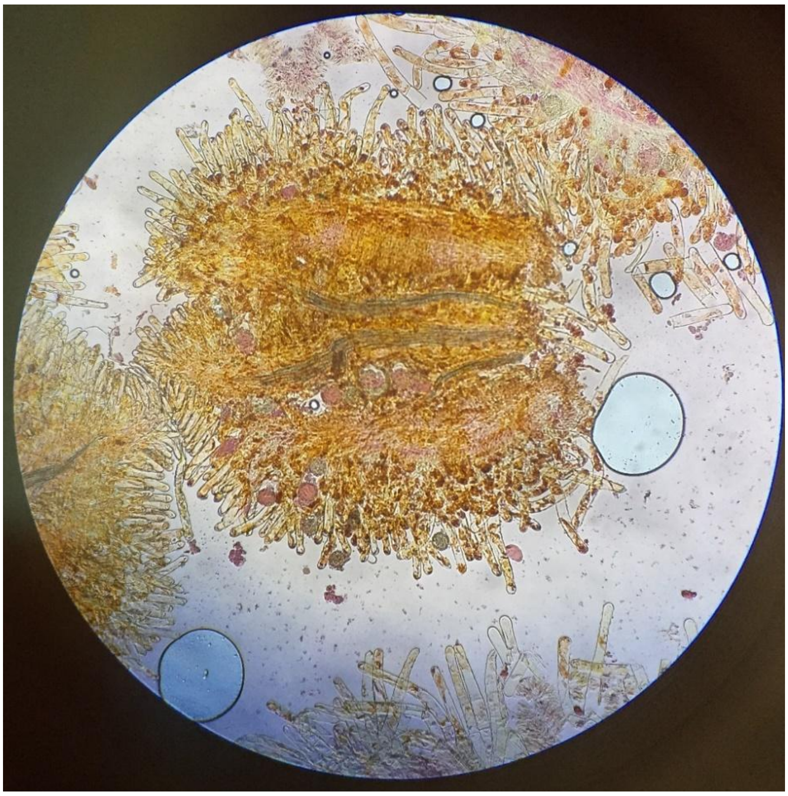

Figure 3. Fertile and sterile pollen grains on microscopic preparation. Magnification x 100

Figure 4. Fertilize grain on microscopic preparation. Magnification x 100

Analysis of the sterility of the marigold pollen grains at various points in Almaty is presented in Table 1. The highest level of sterility of pollen grains is at the collection place (CP) №1 in the Park of First President of the Republic of Kazakhstan and is 48.90%. The level of pollen sterility in marigolds collected at CP №2 at the intersection of al-Farabi Avenue and Kozybayev Street and CP №5 at the campus of the Kazakh National University of al-Farabi is 41.30% and 42.02%, respectively, but there is no statistically significant difference between these points. The level of pollen sterility in CP №6 in the Central Park of Culture and Leisure is 39.44%. The pollen sterility of marigolds collected at CP №3 in the Samal-1 microdistrict and CP №4 at the corner of Satpayev and Baytursynov Streets is 33.90% and 32, 35%, respectively, but there is no statistically significant difference between these points. At the same time, the level of pollen sterility in CP №1 is statistically significantly different from the levels of sterility in CP №3, №4, and №6 ($p<0,05$).

Table 1. Level of fertility and sterility of pollen grains at different places of Almaty city.

Collection place	Address of collection place	Fertile	Sterile	Overall number of investigated pollen grains
		Amount (%)	Amount (%)	
1	Park of the First President of the Republic of Kazakhstan	51,10±3,06	48,90±3,06	266
2	The intersection of al-Farabi Avenue and Kozybaev Street	58,70±6,20	41,30±6,20	63
3	Samal-1 micro district, Zholdasbekov Street	66,10±4,30	33,90±4,30	121
4	The intersection of Satpayev Street and Baitursynov Street	67,65±5,67	32,35±5,67	68
5	Territory of campus of Kazakh National University of al-Farabi	57,98±4,52	42,02±4,52	119
6	Territory of Central Park of Culture and Leisure	60,56±3,35	39,44±3,35	213

Thus, the smallest gametocidal effect of pollutants falls on CP №4, and the greatest – on CP №1. It is worth noting that percentage of sterility does not exceed 50% in any CP.

The results of atomic absorption spectrometry are presented in Table 2. According to the data, amount of lead in the soil did not exceed the threshold limit value (TLV) at all collection points, the amount of manganese exceeded TLV only at CP №5 (1.16 times). The amount of other heavy metals in the soil at all collection points is higher than the TLV.

Table 2. Amount of heavy metals (Pb, Zn, Cu, Cd, Co, Ni, Cr, Mn, Fe) in soil of different collection places.

Heavy metals	Collection places						Threshold limit value
	1	2	3	4	5	6	
Pb	17,27	23,85	22,62	15,68	25,23	21,61	32
Zn	118,6	130,0	119,2	123,10	134,4	150,0	70
Cu	7,75	7,75	6,47	6,91	6,97	7,03	3
Cd	0,59	0,56	1,01	0,59	1,55	0,63	0,5
Co	8,78	11,41	11,53	24,76	12,98	10,74	5,0
Ni	17,95	23,80	10,56	24,20	26,14	20,50	4,0
Cr	13,70	21,12	21,33	12,67	23,07	12,15	3,0
Mn	1077,77	1143,45	1237,82	1082,40	1746,62	961,00	1500
Fe	266,67	280,83	352,38	280,11	295,24	346,67	120
Overall amount of heavy metals	1599,08	1642,77	1797,12	1556,22	2272,2	1530,33	

At CP №1 (territory of Park of the first President of the Republic of Kazakhstan), amount of zinc in the soil exceeded the TLV by 1.69 times, copper – 2.58, cadmium – 1.18, cobalt – 1.76, nickel – 4.49, chromium – 4.57, iron – 2.22 times. At CP №2 (al-Farabi – Kozybayev) the zinc content in the soil exceeded the TLV by 1.86 times, copper – 2.58, cadmium – 1.12, cobalt – 2.28, nickel – 5.95, chromium – 7.04, iron – 2.34 times.

At CP №3 (Samal-1), the amount of zinc in the soil exceeded the TLV by 1.70 times, copper – 2.16, cadmium – 2.02, cobalt – 2.31, nickel – 6.19, chromium – 7.11, iron – 2.94 times.

At CP №4 (Satpayev – Baitursynov) the amount of zinc in the soil exceeded the threshold limit concentration by 1.76 times, copper – 2.3, cadmium – 1.18, cobalt – 4.95, nickel – 6.05, chromium – 4.22, iron – 2.33 times.

At CP №5 (the territory of KazNU) the zinc content in the soil exceeded the TLV by 1.92 times, copper – 2.32, cadmium – 3.1, cobalt – 2.6, nickel – 6.54, chromium – 7.69, manganese – 1.16, iron – 2.46 times.

At CP №6 (territory of the Central Park of Culture and Leisure) the amount of zinc in the soil exceeded the TLV by 2.14 times, copper by 2.34, cadmium – 1.26, cobalt – 2.148, nickel – 5.13, chromium – 4.05, iron – 2.89 times.

Therefore, the studied collection points can be ranked by contamination of the soil as follows: the territory of the KazNU (CP №5) > Samal-1 micro district (CP №3) > al-Farabi avenue – Kozybayev Street (CP №2) > Park of the First President of the Republic of Kazakhstan (CP №1) > Satpayev Street – Baitursynov Street (CP №4) > Territory of the Central Park of Culture and Leisure (CP №6). To determine the relationship between the levels of sterility of pollen and the content of heavy metals in the soil, a correlation analysis was performed (Figure 5-6). The correlation coefficient for copper was r = 0.77, and for nickel, r = - 0.61, which indicates, respectively, a strong positive and average negative correlation between the nickel content in the soil and the sterility level of Tagetes erecta pollen grains. No correlation was observed for the remaining heavy metals.

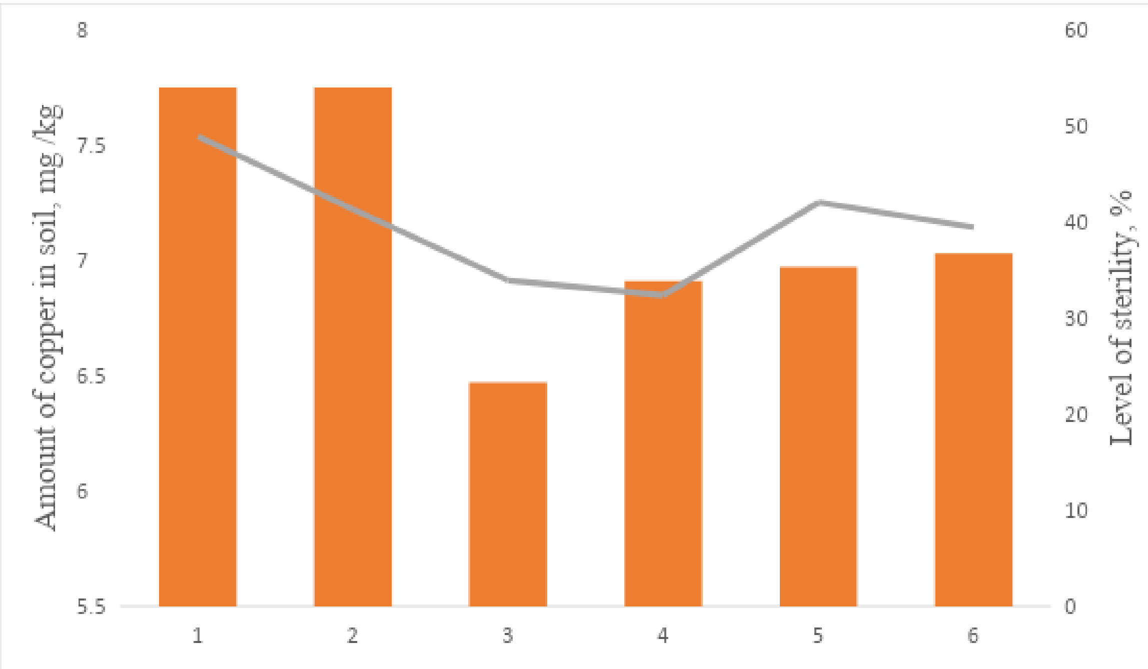

Figure 5. Effect of copper in soil on the level of pollen fertility of marigold.

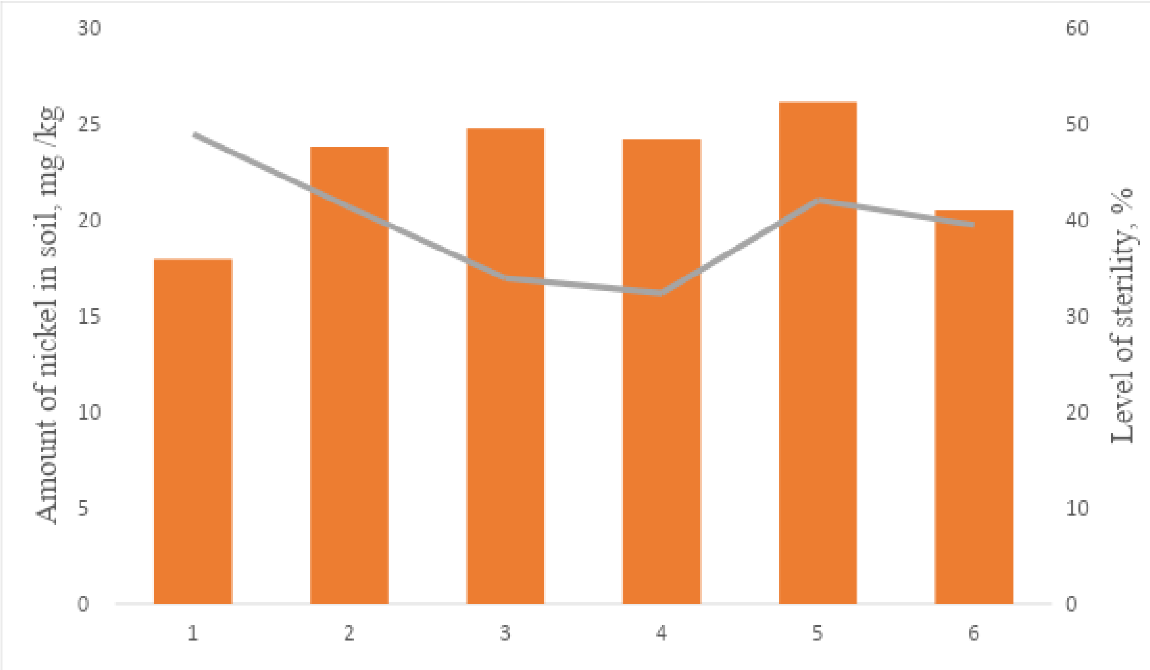

Figure 6. Effect of nickel in soil on the level of pollen fertility of marigold.

During the research, it was found out that marigold pollen grains had an average fertility level, regardless of the area of the city and the content of heavy metals in the soil. This may have occurred due to the presence of other pollutants in the atmosphere. Because of the high level of air pollution, other pollutants – carbon dioxide and sulfur dioxide, could affect the fertility of pollen. For this reason, heavy metals are not the only cause of the observed trend.

Conclusion

A study of the genotoxicity of heavy metals can be carried out using different test systems. Using plants as a test system, it is possible to determine the effect of heavy metals on fertility and plant development and, thus, assess the threats on human health and biodiversity in the country. For residents of Kazakhstan, such studies are especially important, as the developed mining and metallurgical industries pose a threat of contamination by heavy metals. In addition, these studies are important due to the fact that the problem of contamination with heavy metals and their genotoxicity were not covered in Kazakhstani studies in the same way as in foreign ones. Thus, the results of the study revealed that:

- At all collection points the level of pollen sterility does not exceed 50%. According to the level of sterility of marigold pollen, the studied collection points can be ranked as follows: Park of the First President of the Republic of Kazakhstan (CP № 1) > KazNU territory (CP №5) > al-Farabi Avenue - Kozybayev Street (CP №2) > Central Park of Culture and Leisure of Almaty (CP №6) Samal-1 micro district (CP №3) > Satpayev Street - Baitursynov Street (CP №4).
- The amount of heavy metals in the soil at all collection points exceeds the TLV for iron, copper, cobalt, nickel and chromium. Collection points can be ranked by soil contamination with heavy metals as follows: Territory of the KazNU (CP №5) > Samal-1 micro district (CP №3) > al-Farabi avenue - Kozybayev Street (CP №2) > Park

of the First President of the Republic of Kazakhstan (CP №1) > Satpayev Street Baitursynov Street (CP №4) > Territory of the Central Park of Culture and Leisure (CP №6).

The obtained data can be used in biomonitoring of the ecosystem of the Almaty city, as well as analyzing the pollution of the atmosphere and soil in this ecosystem.

For references, footnotes and endnotes, click here.