MONITORING OF THE LIVING ENVIRONMENT THROUGH AIR POLLUTANTS IN THE POLOG FIELD OF THE REPUBLIC OF NORTH MACEDONIA

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Abstract

Introduction: The Republic of North Macedonia and especially the field pit of Polog in terms of geographical position and climatic aspect is considered as a place with different variables such as: temperature, humidity, radioactive exposure, hilly-mountainous relief. The Sharr mountain in the west and the dry mountain in the north, limit the Polog field pit in all directions, such as north-west and south-east, causing the living environment to deteriorate in the winter period due to air, water and land pollutants. With special emphasis, we highlight the winter season when we have an alarming state of air pollution, such as certain regions of the Republic of North Macedonia and especially the region of the field pit of Polog with the cities of Tetovo and Gostivar and the surrounding area.

Recently, the city of Tetova and its surroundings is considered one of the most polluted cities in the country, region and the largest in the world, then this phenomenon of pollution necessarily leads us to unwanted consequences for the population that lives and operates in this region.

As a result of environmental factors such as air pollution and other related factors such as: stress, temperature, food, social genes, mental state and other related factors can lead to disorders in the human body and lead to diseases that attack the nervous and cardiovascular systems, skeletal and thyroid.

Many studies talk about the alarming condition of patients who are affected by these diseases, therefore our paper will focus on the effect of these factors above all on the actual state of pollutants in the Polog region of North Macedonia.

The purpose of the study: This paper aims to investigate the potential causes that can affect the health of the population of the Polog field pit and especially the city of Tetova and its surroundings.

As a potential pollutants of the living environment, the following can be considered: carbon monoxide, carbon dioxide, sulfur dioxide, urban combustion from automobiles, combustion of various physical substances, chemical components from factories, smoke from chimneys from burning oil and coal, combustion from wood in the form of pellets, etc.

Material and working method: In the study, fertilized chicken eggs were taken and incubated in the incubator for 21 days of incubation. The eggs were treated with different doses of air pollutant extract at certain stages of embryonic development. Using the stereomicroscope with macroscopic and microscopic method were analyzed ontogenetic changes in certain stages of embryonic development.

Research results: The results of the research will give a real insight into the situation of the level of pollution of the living environment in the city of Tetovo and its surroundings and in the field pit of Pollog.

The monitoring of the living environment can be followed by the treatment of air pollutants in certain doses in the form of ampoule therapy, and the negative effect of the given dose on the hen's eggs will be observed. All eggs with the highest dose of injection had a stagnated embryonic development, whereas egss with lower dose of injections had underdeveloped embryos.

Conclusion: From the results of the research we can conclude that the potential pollutants from air pollutants can lead to consequences for the population living in this region but also for the living world in general.

These factors can lead to disorders of the nervous system, cardiovascular, and thyroid disorders and other respiratory diseases.

This research will give a real insight into the pollution of the living environment and the possible consequences of this at a local, regional and wid er level.

Keywords: environmental pollutants, dose effect, chicken eggs, stages of embryonic development, health effect.

1. Introduction

North Macedonia, a landlocked country in the south-central Balkans. It is bordered to the north by Kosovo and Serbia, to the east by Bulgaria, to the south by Greece and to the west by Albania and the capital is Skopje. The Republic of North Macedonia is located in the northern part of the area traditionally known as Macedonia, a geographical region bounded to the south by the Aegean Sea and the Aliakmon River; to the west by the lakes of Prespa and Ohrid and to the north by the mountains of Montenegro region near Skopje and the watershed between the basins of the Morava and Vardar rivers. The Pirin Mountains mark its eastern edge. Geologically, North Macedonia consists mainly of ancient heavily folded metamorphic rocks, which in the west have eroded to reveal older granites.

North Macedonia is mostly mountainous, with many peaks rising above the tree line to 6,600 feet (2,000 meters) above sea level. The highest elevation is on Mount Korabi (9,030 feet, or 2,752 meters) on the Albanian border. Near the mountains of Sharri with an altitude of 2748 meters in the northwest, the country is covered with forests. Where this has been cleared (and often in the past overgrazed), thin skeletal soils have undergone dramatic erosion and gullying. There are also some wide and fertile valleys that offer good potential for agriculture.

North Macedonia lies at the intersection of two main climatic zones, the Mediterranean and the continental. Periodically, air passes through mountain barriers to the north and south, bringing dramatically contrasting weather patterns; an example is the cold northerly wind known as the vardareci. In general, it has a moderate continental climate: temperatures average in the 30s F (about 0 °C) in January and rise to the 60s and 70s F (about 20-25 °C) in July. Annual rainfall is relatively light, between about 20 and 28 inches (about 500 and 700 mm). Precipitation of less than 1 inch (25.4 mm) in the driest months (July–August) increases to nearly 4 inches (about 100 mm) in October–November. Due to differences in locality and relief, there can be considerable variation in climate, with eastern areas tending to have milder winters and hotter, drier summers and western (more mountainous) regions with harsher winters.

Numerous environmental contaminants significantly contribute to human disease, affecting climate change and public and individual health, resulting in increased mortality and morbidity. Because of the scarcity of information regarding pollution exposure from less developed nations with inadequate waste management, higher levels of poverty, and limited adoption of new technology, the relationship between pollutants and health effects needs to be investigated more. A similar situation is present in many developed countries, where solutions are only discovered after the harm has already been done and the necessity for safeguards has subsided. The connection between environmental toxins and health needs to be better understood due to difficulties in quantifying exposure levels and a lack of systematic monitoring. Different pollutants are to blame for both chronic and acute disorders.[1]

Chemicals that are harmful to human health and have gotten into the environment due to human activity are called environmental pollutants. Additionally, environmental pollution is caused by natural events like volcanic eruptions. Human activities introduce pollutants by polluting the water, air, and soil. Inhalation, oral absorption, and ingestion are the three main ways that contaminants reach the human body. To indicate the amount of a specific pollutant that is consumed, the word "dose" is frequently employed. The dose is dependent on exposure duration and intensity. Depending on the exposure level, different health effects may result. Although industrialization develops a country, it introduces a large number of pollutants into the environment, which harms the health of those exposed. [2]

In general, hazardous substances from both natural and man-made sources pollute the air. The main sources of contaminants include automobile emissions, power plants, burning garbage, chemical companies, and volcanic eruptions contaminants like sulfur dioxide (SO2), carbon monoxide (CO), nitrogen oxides (NOx), heavy metals, biological contaminants, ozone, tobacco

smoke, etc. are all released into the air that is inhaled When these pollutants are ingested, they interfere with the body's internal functioning, causing diseases like cancer, cardiovascular, reproductive, prenatal central nervous system, and respiratory health issue. Tobacco smoke, which consists of harmful chemicals like benzene, cadmium, arsenic, formaldehyde, and nicotine is responsible for health illnesses. It will cause cancer, not only to the smoker but also affect passive smoker (who is exposed to tobacco smoke and is not a smoker). A person may develop asthma, bronchitis, throat infection, and a burning sensation in the eyes. Exposure to biological pollutants like bacteria, viruses, house dust, mites, cockroaches, and pollen can cause asthma, hay fever, and other allergic diseases, and volatile organic compounds cause eye, nose, and throat irritation, headaches, nausea, and loss of coordination. Prolonged exposure may cause damage to the parts of the body, mainly the liver. Lead exposure can harm the brain and digestive systems, and in certain circumstances, it can result in cancer. Exposure to ozone causes itching in the eyes, burns, may develop respiratory disorders like asthma, and our resistance to colds and pneumonia will be lowered. In winter, children may suffer from respiratory problems from exposure to oxides of nitrogen. Depending on the exposure's type and intensity, the effect may be either short- or long-term. Short-term effects range from irritation of the eye, skin, nose, and throat, coughing, headaches, nausea, and dizziness to severe conditions like asthma, bronchitis, and lung and heart problems. Long-term effects will be neurological, reproductive, respiratory, and cancer.[3]

2. Materials and Working Method

Incubation and injection

In the study, 20 fertilized country chicken eggs (from Drenovec neighborhood, Tetovo) were taken and incubated in the incubator for 21 days. The eggs were treated with air pollutant at certain stages of embryonic development. The eggs hatched on day seven, day fourteen and day twenty one. Using the stereomicroscope with macroscopic and microscopic method were analyzed ontogenetic changes in certain stages of embryonic development.

On the 6th day of incubation the eggs were grouped into 4 groups (3 eggs group 1; 7 eggs group 2; 5 eggs group 3; 5 eggs group 4) and then injected with air pollution extract:

- Group 1: injected with 0.5mL air pollution extract
- Group 2: injected with 1.0mL air pollution extract
- Group 3: injected with 1.5mL air pollution extract
- Group 4: control eggs; were not injected

On different days of incubation development of embryos from all three groups was studied after each egg was hatched. Size and embryo weight, organ development, formation of head, beak, eyes, limbs, feathers were monitored.

The injection method used for this study was injection in the air chamberat the bottom of the egg, where hole was punched with a needle, then different doses of air pollutant extract was injected. The injection area was then disinfected and sealed with tape. Control and experiment eggs were kept in the same incubator (the same environment, temperature and conditions).

Data collection

Incubation, injection and processing of the data for this experiment was done in the period from November 2023 to February 2024. The incubation of the chicken eggs lasted 21 days. On different days on incubations, eggs from the experiment groups were opened, and data was gathered, comparing the results with the embryonic growth from the control group.

The air pollutant extract was collected in the mornings from the doorposts, threshold and entranceway of houses in three regions of the city of Tetovo in North Macedonia.

- at the exit of the Tetovo city in Drenovec neighborhood
- in the center of the city of Tetovo near the Clinical Hospital of Tetovo
- in the village of Recicë e Vogël Tetovo

3. Results

During the incubation of the eggs, the weight was measured at different stages of embryonal growth (Tab.1).

Normal weight loss can be observed in the first week, and then its gradual increase in the following stages of embryonic growth. The injection with different doses of air pollutant extract was carried out on the 6^{th} day.

Table 1. Tabular presentation of specimens during incubation of eggs at different stages of embryonic
development.

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Nr.	Egg	Weight before	Weight on day 14 of incubation (gr)	Dose of Injection	Status	
		incubation (gr)	, O	, v		
1	1K	61.6	55.6	No Injection	Normal development	
2	2K	70.6	61.4	No Injection	Normal development	
3	3K	53.0	49.1	No Injection	Normal development	
4	1E	67.5	62.4	0.5mL	Normal development	
5	2E	68.5	61.3	0.5mL	Underdeveloped embryo	
6	3E	65.2	60.9	0.5mL	Underdeveloped embryo	
7	4 E	61.7	57.6	0.5mL	Underdeveloped embryo	
8	5E	62.1	58.9	0.5mL	Underdeveloped embryo	
9	6E	57.0	53.3	0.5mL	Normal development	
10	7E	65.4	60.8	0.5mL	Underdeveloped embryo	
11	8E	67.2	62.2	1mL	Underdeveloped embryo	
12	9E	65.8	62.4	1mL	Very underdeveloped	
					embryo	
13	10E	60.2	57.1	1mL	Underdeveloped embryo	
14	11E	60.3	56.1	1mL	Very underdeveloped	
					embryo	
15	12E	64.6	59.3	1mL	Very underdeveloped	
					embryo	
16	13E	73.4	68.9	1.5mL	Stagnated development	
17	14E	73.0	67.7	1.5mL	Stagnated development	
18	15E	62.8	58.4	1.5mL	Stagnated development	
19	16E	68.9	64.8	1.5mL	Stagnated development	
20	17E	71.8	67.1	1.5mL	Stagnated development	

Experiment eggs were hatched and then embryonic development in different groups was observed.

In <u>Group 1</u>, we can observe the highest level of embryonic development. This correlates with the dose of injection, where in this group is the lowest (0.5mL of pollution extract)

- Egg 1E: a fully developed chick, no macroscopic issues observed; the chick in healthy and in good condition.
- Egg 6E: the chick is not in the best condition but it macroscopically no issues can be observed.
- Egg 7E: underdeveloped chick; macroscopic issues such as lower weight, size, smaller beak, fewer feathers were monitored.

In eggs of <u>Group 2</u> where the dose is raised to 1.0mL, underdevelopment can be observed in all eggs.

- Egg 10E: underdeveloped embryo; small weight, size, beak can be observed.
- Egg 11E: very underdeveloped embryo; underdeveloped organs, small size & weight of embryo, less feathers than normal can be observed.
- Egg 12E: very underdeveloped embryo; underdeveloped organs, small size & weight of embryo, less feathers than normal can be observed.

In eggs of <u>Group 3</u> we can observe the effects of the higher dose of 1.5mL of pollution extract on the eggs, where development has stagnated.

- Egg 13E: stagnated development; the high dose has completely stopped embryonic development.
- Egg 14E: stagnated development; the high dose has completely stopped embryonic development.
- Egg 21E: stagnated development; the high dose has completely stopped embryonic development(Fig.1).

On days 15-21 of incubation, the development of egg embryos from each group was studied. Embryo weight, organ formation, embryo size, formation of beak, eyes, feathers were monitored.

As expected, on control eggs normal embryonic development is clear and distinct macroscopically (Fig.2).

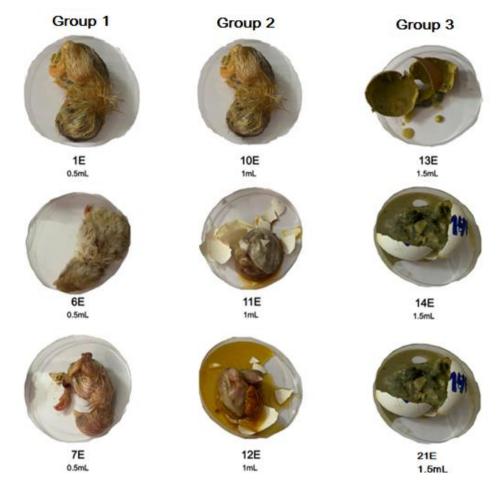
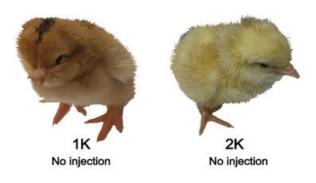


Figure.1 Schematic representation of the different stages of embryonic development of chicken eggs injected with pollutants in different doses in different periods of time from day 1 - 21.

Group 4



Day 21

4. Discussion

Some studies have shown that fertilized chicken eggs have been a great model for laboratory experiments. The use of pollutants in fertilized chicken eggs is not the only time research has focused on different pollutants for embryonic growth, organ formation, external size of the embryo, formation of the head, beak, eyes, feathers, limbs etc.

Chicken embryo has been used as a development model for over 200 years, [4] which allows direct observation and manipulation of developing embryo. Moreover, chicken embryo is a closed system, allowing relatively accurate exposure to toxicants without the interference of maternal effects. In the current study, chicken embryo model was utilized to assess the potential developmental toxicities induced by PM2.5 exposure. PM2.5 was exposed to the chicken embryos *via* air cell injection, by which the PM2.5 was injected onto the air cell membrane without direct penetration into the egg. This method has been demonstrated to elicit comparable results to real-world exposure to environmental contaminants. [5]

Early (embryonic day four, ED4) and late development stages (hatchling chickens) were both investigated for a more comprehensive understanding of the developmental toxicity at different stages of development. For early development assessment, ED4 embryos were used, since the embryos are undergoing organogenesis at this stage, in which the vital organs such as brain, heart, and eyes are visible, but the organogenesis is still going on, and defects may be visible in response to extragenous disruptions. [6], [7], [8]

For late development assessment, hatchling chickens were selected, since most organ systems finished development at this stage.

Animals, like humans, act according to physiological patterns of behavior that are manifested in one or a combination of the following behaviors: social behavior, kinetic behavior, reproductive behavior, feeding behavior, exploratory behavior, hygienic behavior (including excretion, thermoregulation, and comfort behavior), territorial behavior, reactivity, and behavior related to rest and sleep. [9] Each type of behavior is manifested through different activities that are constantly changing: "overt" activities that can be detected by observation, such as walking, sleeping, vocalizations, eating, etc., and "covert" activities that can be detected by instrumental methods (physiological processes related to the functions of the brain, heart, and other organs controlled by the autonomic nervous system), such as fluctuations in blood pressure. [10], [11] [12]

Behavioral changes may also occur as a result of exposure of the organism to pollutants. A pollutant may be a chemical substance (toxic metals, radionuclides, organophosphorus

Figure 2 Presentation of chicken specimens of the control group on day 21 after hatching.

compounds, gasses, etc.) or geochemical substance (dust, sediment), a biological organism or product (bacteria, viruses, etc.), or a physical substance (heat, radiation, sound waves) that is intentionally or accidentally released by humans into the environment and has actual or potential harmful, unpleasant, or nuisance effects. Environmental pollution can be classified as air pollution, water pollution, land pollution, noise pollution, radioactive pollution, light pollution, thermal pollution, and so on. Pollutants are environmental stressors.[13]

Animals are exposed to environmental pollutants just as much as humans, and in some cases even more so. Although the sensitivity of animals to individual pollutants is not the same in all species, almost all pollutants can result in death of the animal, preceded by more or less noticeable changes in behavior, a decline in production, increased susceptibility to stressors and diseases, reduced reproductive success, damage to the respiratory system, neurological problems, etc. Changes in common behavior are the first sign that the animal is uncomfortable in the environment it is in and it should not be ignored. In environments where both people and animals are present, a change in animal behavior may indicate that these conditions are not suitable for people, either. [14]

The results of our research correlate with the data of different world authors regarding this negative phenomenon for the living environment.

5. Conclusions

The results obtained from the experiment give us valuable information about the effects the pollution in the area of Polog field pit can have in chicken eggs and embryonic development.

From all eggs injected with pollution extract we can see negative effects such as underdevelopment in a minor level (lower weight, size of embryo etc.), in a high level (small formation of beak, less feathers in the embryo etc.), or a complete stagnation of the embryotic development. The level varies of the dosage, where the higher dose injections turned out to be lethal and completely stagnate the development.

Meanwhile, factors might have a negative impact that can lead to disorders of the nervous system, cardiovascular, and thyroid disorders and other respiratory diseases is also a topic worth looking into.

Air pollution exposure affects a large population among the world. While its negative health effects are currently being extensively investigated, the developmental toxicities received relatively less attention.

The pollution of the living environment by different pollutants that may be of chemical, physical or biological origin can have an impact with negative consequences for the living environment and diseases that attack humans in particular and the living world in general and disrupt the balance of life and cause imbalance all around the world globe.

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