



Histopathological changes in testes of house sparrow (*Passer domesticus*)

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Abstract

To detect the effect of heavy metals such as lead, nickel, cadmium, copper and zinc in the testis tissue, we used the house sparrow (*Passer domesticus*) as a bio-indicator of pollution by metals. The house sparrows (*Passer domesticus*) were caught in the city of Mitrovica (contaminated area, the "Trepça" foundry is closed since the year 2000), in the town of Drenas (the area near the Ferronickel smelter, also a contaminated area) and in a rural area - control (the village of Ujmirë, a non-contaminated area). In these localities, along the month of May, a total number of 60 sparrows were caught and isolated. After isolation, the sparrows testes were cut by microtome and then tissues were inserted into the staining procedure. Results showed major histopathological changes in the testes of sparrows which were caught in the city of Mitrovica and Drenas compared to the testes histology of the control group (Ujmirë). The testes histopathology includes degenerative changes such as apoptosis and necrosis of the testicular tubes, karyopyknosis and desquamation of the spermatogen epithelium, depletion of the seminiferous tubules, some seminiferous tubules exhibit some small numbers of spermatozooids - an indication of the lack of active spermatogenesis in the testes. From our results we can conclude that the areas in which the study was carried still pose a danger to the living world (biota) and to the people's health.

Keywords: Heavy metals, *Passer domesticus*, testes, histopathology.

Introduction

Environmental pollution is a serious problem in many parts of the world, leading to forest decline, loss in agricultural production and seriously endangering human health. Concern is environmental pollution with heavy metals. Many of these elements produce toxic effects following excessive exposure, while characteristic pathologies can result in deficiencies [1, 2, 3]. Effects of metal or multi metals exposure on the testis are of concern since occupational exposure to certain metals results in impaired reproductive function. Heavy metals, which are essential for normal testicular function, include zinc, manganese and selenium, while those, which are toxic, are lead, cadmium, mercury and cobalt [4]. Our research is done in the city of Mitrovica and Drenas town, while the reference point is taken, village Ujmirë.

The pollution of living environment in Mitrovica still presents an ecological problem (the area near foundry "Trepça" closed since 2000). The main recourses of pollution in Mitrovica, in the past had as a starting point different technological departure (metals foundry, refinery, flotation, factory of accumulations and sulfuric acid battery) of "Trepça" combine and also superphosphates fertilizer factory. The works of these departments have made Mitrovica of the most polluted cities in Europe. The pollution level can be illustrated from some information's by Popovac [5], who find that the quantity of the emitted lead dust everyday from the Trepça foundry in 1979 was 5 to 6 tons. The concentration of lead in air in Mitrovica from the same period was 20-30 µg/m³. Trepça foundry was closed in August 14th 2000 from the United States Forces. The research that was

done from Shehu's [6] analysis of soil taken in the location around Mitrovica had notice high concentration to Pb (23120,0 ppm), Zn (46160 ppm), Cu (3688,0 ppm) and Cd (313,0 ppm). More than 13 years have passed by but the pollution left from Trepça's activities, threatens seriously the local environment and people's health. Drenas town is located 20 kilometres far from Prishtina (capital of Republic of Kosovo) in the West. The smelter (Ferronickel) has operated since 1982. Ferronikeli has three open pit mines: the Dushkaja mine with estimated reserves of 6.2 million tonnes; the Suka mine-0.8 million tonnes and the Gllavica with 6.8 million tones. Ferronikeli smelter is well-known for final production of Ferro- nickel. In metallurgical processing the mineral base is treated by an oxide mineral of nickel (two sources) with the following average chemical structure: Ni+Co=1.2%; Fe=26.0%; SiO₂=47.0%; CaO=2.5%; Cr₂O₃=1.2%; MgO=11.0%. The produced slag has the chemical structure as follow: Ni-0.08%; SiO₂ 55-57 %; MgO 10.0%; Fe total 20%; Ca) 4.0%. The capacity of the smelter in the technological lines (rotating furnace and electrical furnace) is about 12.000 t Ni/year [7]. In recent time, the smelter plant is active and it is known as "New CO Ferrocickel". From the group of heavy metals, Cd and Pb are more serious heavy metals which produce histopathological alterations that include liver damage [8], respiratory dysfunction [9], testicular and ovarian damage [10, 11].

Areas in which the samples are taken (the house sparrows), are characterized by a high concentration of heavy metals, specifically with lead. Different researchers have made various studies with different animals caught in natural populations that were located near lead smelters and the results indicate a positive correlation between the level of contamination and the accumulation of some heavy metals in testicles and different tissues. In previous studies, there was a great interest in the usage of birds (Aves) as defining bio-indicators for environmental pollution by heavy metals.

Birds are much easier to study than other bio-indicator and this makes them ideal for monitoring purposes. The house sparrow (*Passer domesticus*) is a member of Passeridae family with 160-165 mm height, by opening their wings from 210-255 mm. It is characterized with a very strong sense of hearing and a very strong sense of sight and sharp beak. Male sparrows have in the front part of their neck a black tape especially during their time while building their nests. Sparrow historically is known as one of the birds that had a close relationship with people and known as the bird with the highest spread on the planet [12, 13].

Our designation to the usage of the house sparrow population as an experimental model, is based on several advantages that this bird possesses, for example: Its intimacy with the living environment and people's lives; The closest radius (300 meters from the nest) [14]; the effortless opportunity to ensure homogeneous groups as to age and sex; higher metabolism (the number of heartbeats 460 /min at rest, but in cases of flight and danger the heartbeats goes up to 1000 per minute); short life (they live 2-3 years, or up to 7 years - in the living conditions in the cage).

In the area where we have done our research, previously, Elezaj conducted his research, using the Feral Pigeon (*Columba livia*) [15] as a bio-indicator. Different researchers have used different animals to observe the impact of heavy metals to *Pica pica* [16], *Hirundo rustica* [17]. The aim of our research was the histological analysis of the house sparrow testes tissue (*Passer domesticus*) to see the impact of potential changes to heavy metal pollution in the biota.

Materials and methods

Our research is done on the natural individuals of the population of house sparrow (*Passer domesticus*) who were caught in the city of Mitrovica (polluted area), the town of Drenas (area near the Ferronikel smelter, polluted area) and in rural areas - control (Ujmirë village, not a polluted area). In May (2014), in total 60 sparrows / 20 for each locality were caught (10 testicles pairs/sparrows from each locality were isolated). The histological preparation of the testes is done with the standard method according to Shvob [18]. After narcotization and dissection, the sparrows testes had been taken and placed in formalin 4%. These were embedded in paraffin wax. Thick sections (5µ) were obtained by collecting 3 serial sections from the different depths of the wax block until the entire thickness of the testis was cut. These were stained by the haemotoxylin and eosin and examined using the light microscope. The evaluation is done by analyzing tissue histological.

Results and discussion

The results of the histological analysis of the house sparrow testes (*Passer domesticus*), caught in the city of Mitrovica and the town of Drenas, compared with the control group of sparrows (village Ujmirë), are shown in figure 1-3.

Results showed histopathological changes, such as: apoptos and testicular pipe(funnel) necrosis, expansion of interstitial space, karyopyknosis and desquamation of the spermatogen epithelium, depletion of the seminiferous tubules, some seminiferous tubules exhibit some small numbers of spermatozoids - an indication of the lack of active spermatogenesis in the testes of sparrows caught in the city of Mitrovica and Drenas, compared to the sparrows of the control group (Ujmirë). An active spermatogenesis with tubules filled with spermatozoids is noticed in the testes of the control group sparrows, as well as a great number of Leydig cells.

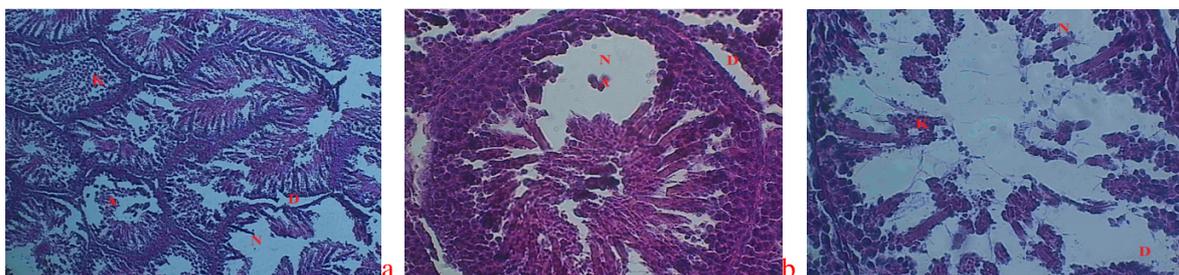


Figure 1 (a,b,c): Microscopic appearance of the testicles sparrow (*Passer domesticus*) in the locality of Mitrovica (month of May). Necrosis (N), Desquamation of spermatogenic epithelium (D), Apoptosis (A) and Karyopyknosis (K).

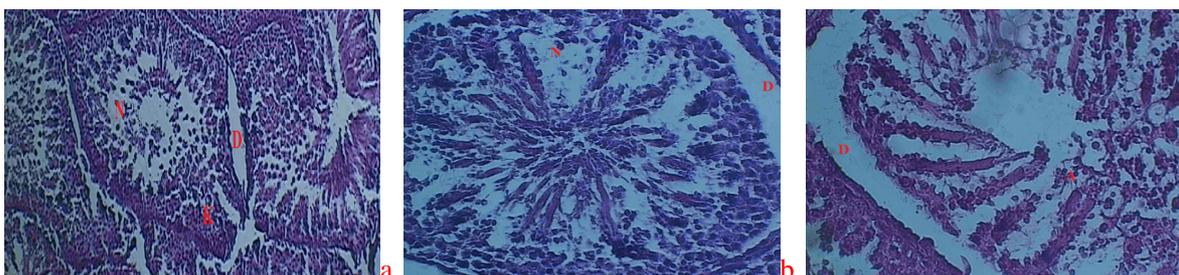


Figure 2 (a,b,c): Microscopic appearance of the testicles sparrow (*Passer domesticus*) in the locality of Drenas (month of May). Necrosis (N), Desquamation of spermatogenic epithelium (D), Apoptosis (A) and Karyopyknosis (K).

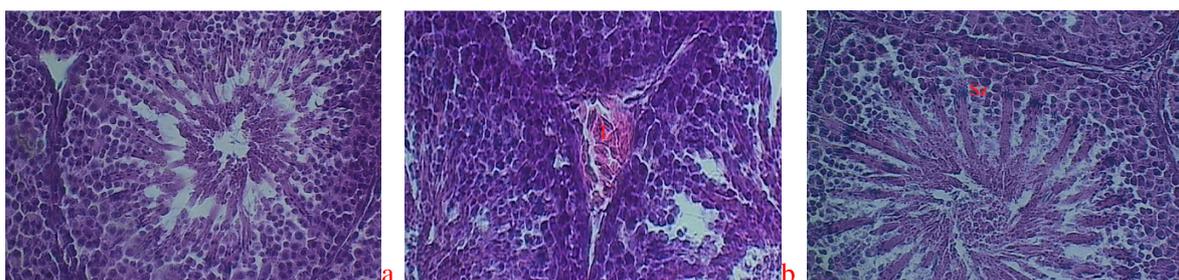


Figure 3 (a,b,c): Microscopic appearance of the testicles sparrow (*Passer domesticus*) in the locality of Ujmir - Control (month of May). Leydig cells (L), Spermatozoa (Sz).

Histopathological changes (expansion of the interstitial space, desquamation of spermatogenic epithelium, atrophy of seminiferous tubules, degenerative and necrotic changes in the germinal epithelium and Sertoli cells, apoptotic characteristics including karyopyknosis) found in the testes of sparrows in Mitrovica and Drenas, are consistent with the results of Rozhaja [19, 20], who have also noticed changes, upon exposure six months of laboratory mouse (Mill Hill) in the foundry yard "Trepça"; in the testes of natural population - terrestrial turtle (*Testudo Hermon*) and the population of vineyard snail (*Helix pomatia*) in factory smelter of lead and zinc

"Trepça" in Mitrovica. Degenerative changes are found in the testes of sparrows (*Passer domesticus*) in Mitrovica and Drenas are consistent with the results of Selimi [21], who found changes, mainly same in the testes of the feral pigeon (*Columba livia*) in Mitrovica.

Histopathological changes, observed in the testes of sparrows (*Passer domesticus*), are consistent with the findings observed in laboratory rats [22, 23] experimentally exposed with lead poisoning, as well as those of patients in cases plumbism [24]. Our results are also consistent with Kendall's results [25], which also finds approximately the same changes in the testes of bird (*Streptopelia risoria*), in the case of lead poisoning. In accordance with our results are experiments from Ghelberg [26], that were conducted in mice (albin), who after treatment with acetate Pb for 154 days. Their findings showed for histopathological changes; reduction in size of seminiferous tubule, the basic membrane separation, reduction of cellular components and spermatocyte lesions. They also found that apoptosis and necrosis cell in tubule testicle, comes from the increasing of lead neurotoxicity. The findings of the present work are basically agreement with investigations conducted on the mice and pheasants (*Phasianus colchicus*) [27, 28] that were treated with Pb (PbNO₃) and Cd, who found empty seminiferous epithelium, spermatogen apoptosis in cells. High index of apoptotic cell death in seminiferous tubule, ascertain Adhikuri [29] who treated mice, experimentally with lead (20 mg/kg of body mass for two weeks). Recent studies show that mitochondria are involved in processes that cause cell death during intoxication with chemicals. One of the main factors of apoptosis, are reactive species of oxygen (ROS), which are produced as a result of the action of metals. Thus, mitochondria are the source of production and target of ROS's action [30]. Due to increased ROS's, increases mitochondrial membrane permeability, followed by damage to the respiratory chains [31]. Physiological death (apoptosis) and accidental (necrosis) of the cells, as the process takes place in two steps: In the first step, the development of many physiological and pathological processes increase the mitochondrial membranes permeability, which release apoptogenic factors outside the membranes, thus, become the loss (decay) of the electrochemical gradient in the inner mitochondrial membranes. Permeability loss causes forming of multiprotein complexes, which are in contact with the inner membrane and the outer mitochondrial.

In the second step, as a consequence of mitochondrial dysfunction (protein collapse, rupture of the respiratory chain, increased superoxide anion, decrease of new mitochondria, flow of calcium and glutathione matrix, and digestion of the protein inter membrane), occur disruption (destruction) of plasma membrane integrity (necrosis), where becomes the activation of the apoptogenic protease with mitochondrial proteins (Cytochrome c, factor that induces apoptosis), and secondary activation of endonuclease - apoptosis [32].

Conclusions

Histological analysis of the house sparrows testes (*Passer domesticus*) caught in the city of Mitrovica and the town of Drenas indicate obvious pathological changes, such as apoptosis and necrosis of the testicular tubules, karyopyknosis and desquamation of the spermatogen epithelium, depletion of the seminiferous tubules, some seminiferous tubules exhibit some small numbers of spermatozooids, presenting an indication of the lack of active spermatogenesis in the testes.

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