

The purpose of the study was to examine the state of soil cover of urban areas with high anthropogenic load (on the example of Gomel) using plant test systems.

For the study used aqueous extract of the soil, the breed of Gomel. Was used the method of *Allium*-test. Were taken into account indicators such as mitotic index, germination energy, the frequency of common aberrations (lagging chromosomes, ahead of a chromosome, chromosomal bridge).

In the experiment, it was shown that combination of chemical elements contained in water extracts inhibited the growth of roots, reduce mitotic index of meristem cells and contribute to the occurrence of chromosomal damage.

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## **IMMUNOFLUORESCENCE AS A METHOD OF ENVIRONMENTAL IMPACT ASSESSMENT ON CELL STRUCTURE**

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Recent advances in fluorescence microscopy allow us to estimate the impact of environmental factors on the molecular level. The opportunity to study different types of cells allows detecting the disorders caused by environmental factors. Impact harmful environmental factors such as radiation can cause malignant tumors in various organs and tissues. Using fluorescence helps in the investigation of the molecular mechanisms of occurrence and development of pathological processes, the effects on the body of biologically active substances.

Currently widely used type of fluorescence is immunofluorescence. Immunofluorescence is a technique used for light microscopy with a fluorescence microscope. This technique uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualization of the distribution of the target molecule through the sample. It is used fluorescent dyes as a markers of monoclonal antibodies (FITC (Fluorescein isothiocyanate), TRITC (Tetramethylrhodamine-5-(and 6)-isothiocyanate), Cyanines (Cy2, Cy3, Cy5 and Cy7), Alexa Fluor).

In fluorescence microscopy not only proteins structures are of interest but also nucleic acids, lipids and etc. DAPI (4',6-diamidino-2-phenylindole) as DNA stains and family of Hoechst dyes are capable to make a difference between DNA and RNA without previous manipulation is Acridine Orange. MitoTracker is used as a cell permeable dye with a mildly thiol-reactive chloromethyl moiety for observation of mitochondria. For staining of the endoplasmic reticulum (ER) are used DiOC6(3) or ER-Trackers. ER-Tracker Green and Red are BODIPY (boron-dipyrromethene) based dyes which are linked to glibenclamide – a sulfonylurease – which binds to ATP sensitive potassium channels exclusively resident in the ER membrane. Recently it is possible to stain special membrane regions like lipid-rafts.

These cholesterol rich domains can be visualized by using NBD-6 Cholesterol or NBP-12 Cholesterol amongst others (Avanti Polar Lipids).

Fluorescent Speckle Microscopy images of actin and microtubules in the lamellae of living epithelial cells were able to observe microtubules and the binding of microvesicles to the membrane (labeled with X-rhodamine tubulin). The spatial and temporal resolution in immunofluorescence is used for studying of dynamics of actin and the actin connected proteins near a plasma membrane in many researches of an endocytosis. Taxol conjugates are used for endpoint assays of cytoskeletal behavior in live cells to provide intense staining of polymerized tubulin. Also, immunofluorescence is applied to track the movement of individual vesicles and exocytosis, which turned out to be a bit more complicated than previously thought. With the help of this method investigate the transmission of signals through the membrane. Immunofluorescence method is also used to study the dynamics of adhesion molecules. It is possible to localize specific DNA sequences in chromosomes or determine spatial-temporal characteristics of gene expression in cells or tissues.

Due to the wide range immunofluorescence dyes, high-affinity monoclonal antibodies used for the immunohistochemical analysis, there appeared the possibility of identifying pathological changes in cells more efficiently.

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## **ANALYSIS OF RECOMBINANT HUMAN ERYTHROPOIETIN BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY**

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Erythropoietin is a glycoprotein hormone that stimulates erythropoiesis through controlling the proliferation and differentiation of the erythroid progenitors. EPO carries out the most important biological functions, concerning it was the first cloned factor of hemogenesis. Human recombinant erythropoietin rhEPO is widely used to treat different types of anemia. Also, it is well-known as a drug enhancing endurance in athletes. In this connection, erythropoietin was banned in some sports by WADA since 1989.

Thus, great importance is development of a method to differentiate between endogenous and exogenous EPO origin. This is due to the fact that about 40% of human erythropoietin molecule weight is compiled by oligosaccharide chains attached to the polypeptide in three N-glycosylation sites (Asn 24, 38 and 83) and one O-glycosylation site (Ser 126). Composition and structure of oligosaccharides chains play critical role in biological activity of EPO. While polypeptide chain is genetically controlled, oligosaccharides chains are result of post-translational modifications which differ in various species and tissues.