

PLOVDIV UNIVERSITY "PAISII HILENDARSKI"

Abstract of the materials under Art. 76 of PRASPU of assoc. prof. Elenka Stoilova Georgieva, PhD

regarding my participation in a competition for the occupation of the academic position "professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological sciences, scientific specialty Morphology announced in the State Gazette no. 92 of 18.11.2022

**Compliance with the conditions for holding the academic position
"professor" under Art. 76 of the Regulations for the Development of the
Academic Staff of the PU "P. Hilendarski"**

Since 1994, I have been a teacher in the Department of "Developmental Biology" at the Faculty of Biology of the Polytechnic University "P. Hilendarski". I obtained the ONS "Doctor" in 2005, and from 2010 until now I have been an associate professor at the same department.

In connection with my participation in the competition for the academic position of "professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences (Morphology), announced in the State Gazette no. 92 of 18.11.2022, I present a total of 47 scientific works, which were not used in the procedures for acquiring the Doctorate and for holding the academic positions of chief assistant and associate professor.

In accordance with the minimum national requirements of the RASRD and the Regulations for the Implementation of the RSARD, the attached scientific works are classified as follows:

- monographic work for indicator B3 – 1 pc.
- scientific works for indicator G7 – 24 nos. (Q1– 4 pcs.; Q2– 4 pcs.; Q3 – 7 pcs.; Q4 – 9 pcs.), of which with an impact factor – 19 pcs. (total impact factor – 34.812)
- chapter from a book to indicator G8 – 1 pc.
- scientific works outside indicator G7 – 19 nos. (referenced in WebSci and Scopus, without Q – 11 items; scientific works outside WebSci and Scopus – 8 items)
- textbooks – 2 pcs.
- manual – 1 pc.

The scientific works are published in English, with the exception of the presented monograph, book chapter, 2 textbooks and 1 study guide, which are in Bulgarian.

All scientific works, with the exception of the monograph, the textbooks and the manual, are published in co-authorship, in which I am the lead author in a total of 26 scientific works, which are distributed as follows:

- in articles for indicator G7 and G8 – 18 pcs.
- in articles outside indicator G7 – 8 pcs.

Total impact factor - 34.812

Total SJR – 8,871

Total number of citations - 407

Total number of citations in journals indexed in WebSci and Scopus – 352

Annotation of the monographic work
of assoc. prof. Elenka Georgieva Stoilova Georgieva, PhD
for indicator B3 in accordance with the minimum national requirements of the
Law on the Development of the Academic Staff in the Republic of Bulgaria
and the Regulations for the Implementation of the Law on the
Development of the Academic Staff in the Republic of Bulgaria.

B3. Elenka Georgieva, 2022, Application of dietary supplement PARA® in men with sub/infertility and men with varicocele, FastPrintBooks publishing house, Plovdiv, 2022, p. 282. ISBN 978-619-236-411-3

Abstract. The study addresses a worldwide problem related to human infertility. Impairment of fertility is observed in both men and women. Approximately half of the reproductive problems of childless couples are the result of male infertility - the so-called "male factor". Unfortunately, in recent years, male infertility is of increasing importance. The causes of male infertility are often complex. They can be under the influence of disorders that have occurred in the body, as well as under the influence of external factors of the environment that affect the reproductive processes directly or indirectly. Indirectly or partially, as additional factors and with a negative influence on the development of infertility, can be lifestyle features, such as intake of: alcohol, smoking, drugs, hazards in the environment and professional environment, social and psychological aspects and others.

One of the common causes of sperm abnormalities is the diagnosis of varicocele. The main method of treatment of this common male pathology is through surgical intervention - varicocele. The availability of conflicting data regarding the effect of varicocele and the probability of pregnancy leads to the idea of including in the treatment and other additional therapeutic approaches.

In addition to the correct diagnosis, an important point in solving male fertility problems is the application of adequate and timely treatment to increase and improve the chances of pregnancy in the partner (spontaneously or with assisted reproductive techniques). There are many controversial issues regarding treatment methods for men with sub/infertility. One of the least invasive methods of improving male fertility is the use of nutritional supplements. In recent years, there has been increasing interest in their inclusion as a therapeutic approach, and especially in multi-component nutritional supplements. It is believed that taking complexes of micronutrients, antioxidants and vitamins could have a strong and relatively quick positive effect on the level of male fertility. Research in this area is currently very popular and such data - valuable to reproductive specialists.

Annotation of the scientific works
of assoc. prof. Elenka Georgieva Stoilova Georgieva, PhD
to indicator G7 in accordance with the minimum national requirements of the
Law on the Development of the Academic Staff in the Republic of Bulgaria
and the Regulations for the Implementation of the Law on the
Development of the Academic Staff in the Republic of Bulgaria.

G7_1. Velcheva I., A. Arnaudov, E. Georgieva*, P. Atanasova. 2012. Influence of copper on the hepatic morphology of the Gibelio carp (*Carassius gibelio*). Journal of Environmental Protection and Ecology, 13(13A): 1928-1932.

ABSTRACT. The scientific publication includes a study that addresses a worldwide problem related to establishing the influence of heavy metals, and specifically copper, on the histostructure of the hepatopancreas in silver caracuda (*Carassius gibelio*). The effect of sub-lethal concentrations of copper sulfate ($\text{CuSO}_4 \times 5\text{H}_2\text{O}$) (0.1, 0.5, 1.0 and 2.0 mg l⁻¹) was investigated for 96 hours. Histopathological changes associated with degenerative disorders in hepatocytes, as well as necrotic changes, were found. Disturbances in hepatic blood circulation associated with hypertrophy of vascular walls, increased erythrocyte diapedesis in the area of hepatic sinusoids, as well as hyperemia of blood vessels were detected. Changes were observed in the shape of the nucleus, which was most often elongated. Bean-shaped, snowflake-shaped, or other nonspecific-shaped nuclei have also been found. A clear gradation of degenerative changes with increasing test concentration was found. Along with them, compensatory changes have been established, which occur even at the lowest concentration and are also gradual. The negative impact of sublethal concentrations of copper on the liver parenchyma, with short-term exposure, as well as a directly proportional dependence of the degree of manifestation with increasing test concentrations, is proven. Findings of such changes indicate that the changes we observed are not specific to copper toxicity, but may serve as evidence of its negative effect in the target organ.

G7_2. Georgieva E.*, I. Velcheva, A. Arnaudov, P. Atanasova. 2012. Study the influence of copper on some indices of the hepatic homeostasis of the Gibelio carp (*Carassius gibelio*). Journal of Environmental Protection and Ecology, 13(13A): 1902-1906.

ABSTRACT. The scientific publication investigates the effect of the influence of concentrations of copper sulfate ($\text{CuSO}_4 \times 5\text{H}_2\text{O}$) (0.1, 0.5, 1.0, 2.0 mg l⁻¹) on the morphological and some biochemical parameters of the liver of silver carp (*Carassius gibelio*). The concentration of 0.1 mg l⁻¹ is below the MPC of concentration according to the Bulgarian standards for copper waters. The degree of morphological exposure of glycogen in the cytoplasm of hepatocytes, as well as the content of lipids and glucose in the blood serum, was determined on frozen sections using the Schiff reaction. A simultaneous increase of glycogen in hepatocytes, as well as lipids and glucose in the blood, was found. The role of blood lipid elevation in the maintenance of liver homeostasis in silver caracuda was discussed. It is suggested that changes in

blood lipid concentration can be used as a biomarker for the purpose of environmental monitoring of copper-contaminated waters.

G7_3. Atanassova P., P. Hrishev, M. Orbetzova, P. Nikolov, J. Nikolova, E.Georgieva. 2014. Expression of leptin, ngf and adiponectin in metabolic syndrome. Folia Biologica (Kraków), 62(4): 301-306.

ABSTRACT. Adipose tissue secretes a variety of adipokines involved in the regulation of energy metabolism and insulin resistance. Metabolic syndrome corresponds to a clinical condition in which white adipose tissue is characterized by an increased production and secretion of inflammatory molecules which may have local effects on adipose tissue physiology but also systemic effects on other organs. The aim of this study was to assess the expression of leptin, NGF and adiponectin in women with metabolic syndrome compared to healthy controls. Plasma leptin, NGF and adiponectin levels were measured by the ELISA method. Leptin and NGF immunohistochemical expression was analyzed in subcutaneous adipose tissue. The results indicated that in women with metabolic syndrome waist circumference, body mass index, HOMA index, glucose, total cholesterol and triglyceride levels were significantly increased in parallel with over-expressed plasma levels of leptin and NGF and decreased adiponectin. The immunohistochemical expression of leptin and NGF was very strong. In conclusion, this is the first study demonstrating a complex of immunochemical and immunohistochemical expression of the key adipokines including leptin, NGF and adiponectin in women with metabolic syndrome. Locally-produced pro-inflammatory adipokines probably contribute to the etiopathogenic mechanisms of metabolic syndrome associated with obesity, insulin resistance, cardiovascular disorders, and hypertension. Leptin, adiponectin and NGF can be used as biomarkers to predict MetSyn and its risks in adolescents, as well as new targets for new therapies.

G7_4. Nikolov P., J. Nikolova, M. Orbecova, T. Deneva, L. Vladimirova, P. Atanasova, P. Hrishev, E. Georgieva*. F. Nikolov. 2015. Flow mediated vasodilation and some biomarkers of endothelial activation in pre-hypertensive objects. The West Indian Medical Journal, 151402.

ABSTRACT. Pre-hypertension is a precursor of hypertension. Endothelial dysfunction is the key element for early prediction of cardiovascular events. We investigated whether flow mediated dilation, a non-invasive method for assessment of endothelial function, is decreased and if there is a parallel with some biomarkers of endothelial dysfunction. 103 patients with pre-hypertension at the age $43,5 \pm 6$ years, were enrolled. Weight, body surface area, waist, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, plasma glucose were followed up for each patient, indicating statistically higher values in the pre-hypertensive subjects. Flow mediated dilation was reduced when compared to our control data from healthy volunteers. It was in parallel with асиметричен метил-аргинин (ADMA) и серумната васкуларна клетъчна адхезионна молекула (sVCAM-1). There were no significant differences in

sICAM-1. Pre-hypertension objects demonstrated reduced flow mediated dilation and significantly changed ADMA and sVCAM-1. Intima-media thickness didn't show any significant differences between pre-hypertensive and healthy objects. In conclusion, there is a correlation between clinical chemical biomarkers, flow mediated dilation, endothelial dysfunction and pre-hypertension which confirms their role as a predictor of pre-hypertension and cardiovascular disorders and as a challenge for primary prevention.

G7_5. Penkova N., P. Atanassova, E. Georgieva, M. Chilikova. 2017. Ghrelin as an ontogenetic factor and a gastrointestinal hormone in the prenatal and postnatal period in rat. IOSR Journal of Dental and Medical Sciences, 16(3) 77-90.

ABSTRACT. Ghrelin is a recently discovered hormone. It was mentioned for the first time by Masayasu Kodzima and associates in 1999. Despite the numerous studies concerning ghrelin, there are still issues to consider. So far there is no definite answer to the question which type enteroendocrine cells produce this hormone. A not less debatable point is the question on the appearance of differentiated ghrelin-producing cells in the prenatal period. So far the earliest occurrence of ghrelin-producing cells in rats is registered in foetal stomach 18th gestation day. The aim of our research is to ascertain the occurrence of ghrelin-producing cells in the developing gastrointestinal tract of a rat during the embryonic, foetal and early neonatal period, to study their manifestations and relations to the differentiating elements of the gastro-intestinal wall. The material for study is of white Wistar rats. We studied rat embryos 8th - 11th gestation day; rat embryos 12th - 15th gestation day; rat fetuses aged 15th -20th gestation day; fragments of gastrointestinal tract of one-day- old rats. We performed an immunohistochemical study on the ABC method with primary ghrelin antibody and primary antibody of ghrelin receptor GHS. We found presence of ghrelin-producing cells in the endoblastic epithelium of rat embryos 12th gestation day and fetuses 16th gestation day, as well as in the epithelial lining and glands of the stomach and small intestine of one-day-old rats. A ghrelin receptor is expressed in the same periods in endoblastic and myoblastic cells of the developing digestive tube in embryos and fetuses, as well as in the epithelial lining and glands of the stomach and small intestines of newborn rats. Ghrelin-producing cells in the gastro-intestinal tract are differentiated as early as the embryonic development of rats. The presence of ghrelin receptors in the endoderm and myoblast of the developing gastrointestinal tube during the embryonic and foetal periods presumes the participation of ghrelin as an inductive signal in the complex processes of cellular proliferation and differentiation. The presence of ghrelin receptors in the mucosa of the stomach and small intestine after birth reveals the ability of ghrelin to participate directly in the regulation of the local processes of the gastro-intestinal wall (secretion, motor and sensory function) without the mediation of the growth hormone.

G7_6. Yancheva V., E. Georgieva, S. Stoyanova, V. Tsvetanova, K. Todorova, I. Mollov, I. Velcheva. 2018. Short and long-term toxicity of cadmium (Cd) and polyaromatic hydrocarbons (PAHs) on zebra

mussel (*Dreissena polymorpha* Pallas, 1771). *Acta Zoologica Bulgarica*, 70 (4): 557-564.

ABSTRACT. This study was designed to examine the possible negative effects, which cadmium (Cd) and polyaromatic hydrocarbons (PAHs) could have on the lysosomal membrane stability in haemocytes of the invasive mollusk zebra mussel (*Dreissena polymorpha*) by applying the neutral red retention assay (NRRA). The mussels were exposed to different concentrations of Cd and PAHs in laboratory conditions for 96 hours (acute exposure) and 31 days (chronic exposure). These are considered as priority substances in surface waters according to Directive 2008/105/EC. We found lysosomal membrane destabilisation in all mussels treated with Cd and PAHs, including concentrations, which were lower than the allowable ones. In addition, we determined a trend of lower retention time in the mussels treated with Cd as compared to the ones treated with PAHs, although these differences were not significant ($p > 0.05$). Our results confirmed that the neutral red retention assay could be used as a cheap, fast and reliable biomarker for Cd and PAHs effects on freshwater mollusks and that zebra mussel could be suggested as a freshwater bioindicator for water contamination. However, further studies are required in order to better understand the negative effects of Cd and PAHs on this bivalve species.

G7_7. Yancheva V., S. Stoyanova, E. Georgieva, I. Velcheva. 2018. Mussels in ecotoxicological studies – are they better indicators for water pollution than fish? *Ecologia Balkanica*, 10(1): 57-84.

ABSTRACT. EU Member states are required to apply the EU Water Framework and its Daughter Directives in order to achieve Good Environmental Status (GES) for all 11 qualitative descriptors by 2015 in all water bodies for a list of priority and specific pollutants. Therefore, environmental indicators and biological-effect techniques have to be carefully selected for the management of chemicals in the aquatic environment and for developing an integrated framework. The most commonly applied biological-effect tools are measures of the biochemical and physiological state of selected organisms, such as mussels or fish. The present article provides basic information on the EU Water Directive, the essence of biomarkers, and outlines why mussels may be the better choice of indicators in toxicological research and monitoring programs in order to study the impact of contaminants in water ecosystems.

G7_8. Todorova K., I. Velcheva, V. Yancheva, S. Stoyanova, P. Dimitrova, S. Tomov, E. Georgieva* E. 2018. Interactions of Pb with other heavy metals (Cd, Ni and Zn) and toxic effects on gills histological structure of common carp (*Cyprinus carpio* Linnaeus, 1775). *Acta Zoologica Bulgarica*, 71(1): 95-102.

ABSTRACT. We performed a laboratory experiment in order to study the toxic effects of five different concentrations of single lead (Pb) and its interactions with other heavy metals, cadmium (Cd), nickel (Ni) and zinc (Zn), on the gills of common carp

(*Cyprinus carpio* Linnaeus, 1775). Cadmium, Ni and Pb are considered as priority polluting substances in surface waters. A short-term experiment (96 h) in laboratory conditions was performed. The fish were treated with decreasing concentrations of single Pb as well as with its combination with Cd, Ni and Zn. Then we examined the fish gill histological changes and the degree of expression of each histological alteration. Several alterations were observed, i.e. lamellar epithelium lifting, edema, proliferation of the stratified epithelium and cartilage tissue, fusion and degeneration in the gill epithelium as well as vasodilatation and aneurysms in the gill blood circulatory system. The alterations were more pronounced for the single Pb-exposed groups than for its combination with the other tested heavy metals. Overall, the study has contributed to clarify the toxicity of single Pb as well as its mixtures (Pb²⁺+Cd²⁺, Pb²⁺+Ni²⁺ and Ni²⁺+Zn²⁺) and the interactions between the metals on the commercially important freshwater common carp. The results of the study are significant in clarifying the debatable issues regarding the impact of the combination of heavy metals on target organs, which is commonly distributed in freshwater and marine water basins.

G7_9. Yancheva V., I. Velcheva, E. Georgieva*, Mollov I., Stoyanova S. 2019, Chlorpyrifos induced changes on the physiology of common carp (*Cyprinus carpio* Linnaeus, 1785): A laboratory exposure study. *Applied ecology and environmental research* 17(2): 5139-5157.

ABSTRACT. The present study was designed to study the effects of the pesticide chlorpyrifos (CPF) on the physiology of the economically important fish species common carp (*Cyprinus carpio* Linnaeus, 1785) by applying a biomarker approach. This pesticide is considered as a priority substance in surface waters according to Directive 2013/39/EU of the European Parliament and of the Council. The fish were treated with decreasing concentrations of CPF for 72 hours (acute exposure) and the histological structure of gills and respiration rate were examined. Changes in vasodilated blood vessels in the primary and secondary gill lamellae were monitored. Degenerative necrotic disorders, as well as hypertrophic and hyperplastic growths of the gill epithelium, are also shown by degree of manifestation. The established histological changes are associated with the deviations in the respiration of the experimental fish. In sum, we found pronounced alterations in the gill structure and changes in the respiration rate index, regardless of the applied pesticide concentrations which indicates its negative effects on non-target aquatic species such as common carp. The results from such studies could be incorporated in the legislation to prevent water contamination in areas with intensive agricultural practices by applying biomarkers, and an update could also be initiated on the maximum permissible concentrations of CPF in surface waters.

G7_10. Yancheva V., I. Velcheva, E. Georgieva*, S. Stoyanova. 2019. Bioaccumulation of polyaromatic hydrocarbons (PAHs) and cadmium (Cd) and its toxic effects on zebra mussel *Dreissena polymorpha*

(Pallas, 1771) (Bivalvia: Dreissenidae). Acta Zoologica Bulgarica, 71(4): 567-574.

ABSTRACT. The possible negative effects of polyaromatic hydrocarbons (PAHs) and cadmium (Cd) on the lysosomal membrane stability (LMS) in haemocytes of the invasive mollusc zebra mussel (*Dreissena polymorpha*) were studied by applying the neutral red retention time assay (NRRT). In addition, the process of bioaccumulation of PAHs and Cd in the gills of zebra mussel was examined and the bioaccumulation factor (BFA) was calculated. The mussels were exposed to different concentrations of Cd and PAHs in laboratory conditions for 96 hours (acute exposure) and 31 days (chronic exposure). Data are presented on the limit of detection and limit of quantification of organics according to the method used. Average values of the main physical and chemical properties of water, as well as bioaccumulation in the short-term and long-term experiment. We found higher toxicant concentrations at the 24th h as well as on the 31st day compared to the other tested time periods. These results were linked with the faster lysosomal membrane destabilisation in all mussels treated with Cd and PAHs in the beginning of the experiment.

G7_11. Yancheva V., Velcheva I., Georgieva E.*, Stoyanova S. 2019. Periodic Acid - Schiff (PAS) reaction in fish liver exposed to fungicide contamination: A possible histochemical biomarker. Ecologia Balkanica, 11(1): 1-10.

ABSTRACT. The present work aimed to study the negative effects of fungicide contamination on the liver of *Hypophthalmichthys nobilis* (Richardson, 1845) by applying the Periodic acid – Schiff reaction (PAS). The fish were treated with increasing and real applicable pesticide concentrations in agriculture prepared according to the guidelines of the producer for a total acute period of 96 hours. Overall, we found that the intensity of the PAS staining increased proportionally with the increasing of the tested fungicide. Based on the obtained results, we consider that the PAS-staining could be successfully applied as a biomarker in toxicological research. In addition, as fungicide studies are in general less compared to the other pesticide groups, we also consider that these results could be used in future risk assessment and monitoring programs, as well as better agricultural activities.

G7_12. Stoyanova R., S. Tomov, E. Georgieva*, P. Atanassova, I. Dechev, V. Yancheva, S. Petrova, S. Stoyanova. 2019. Influence of exogenous factors on the maturation levels of spermatozoa chromatin in sub/infertile men treated with nutritional supplement PAPA®. Ecologia Balkanica, 11(1): 179-189.

ABSTRACT. Changes in the hereditary information during the nucleus maturation of the sperm cells can occur under the influence of exogenous or endogenous factors. Exogenous factors could be linked with unfavorable working environment or bad habits. In the recent years nutritional supplements have been used

for male infertility treatment. In addition, they can improve the morpho-physiological status of the sperm. The present study aims to investigate the effect of nutritional supplement PAPA on the spermatozoa chromatin maturity level by using an Aniline Blue staining test (aniline blue staining - AB) in sub/infertile men exposed to exogenous factors.. A group of men (n = 88) was examined after their written agreement and a completed questionnaire. The tested group with established diagnoses of asthenoteratozoospermia, oligoasthenoteratozoospermia, astenoteratozoospermia with hypovolemia, oligoasthenoteratozoospermia with hypovolemia took orally PAPA® supplement for three months. All patients were tested for DNA damage by an AB test twice before and after the treatment. The patients in the tested group were divided based on the type of harmful work environment (I-IV) and harmful habits (smoking I-III). The results showed that after the treatment with a nutritional supplement PAPA®, the levels of condensation or maturity of sperm chromatin increased by 16.11%. In addition, connections between the effect of treatment and the conditions of the harmful working environment, as well as the amount of received tobacco by the smokers were found. A better effect of the treatment in the tested group with the absence of harmful working environment and non-smokers was found. Therefore, the lowest treatment efficacy was found in the patients who are chronically exposed to occupationally harmful environment, associated with increased body and scrotal temperature (seated workers in factories, ect., drivers, welders, bakers, founders, heavy physical labour workers). On the other hand, a negative connection between the smoked cigarettes per day and the effect of the food supplement in the tested group of men with harmful habits, such as smoking was established.

G7_13. Stoyanova S., K. Nyeste, E. Georgieva*, P. Uchikov, I. Velcheva, V. Yancheva. 2020. Toxicological impact of a neonicotinoid insecticide and an organophosphorus fungicide on bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845) gills: a comparative study. North-Western Journal of Zoology, 16(1): 1-10.

ABSTRACT. The main aim of the present study was to compare the toxicological effects of a fosetyl-Al and fenamidone based fungicide and a thiamethoxam based insecticide on the gill histological structure of bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845) in a short-term laboratory conditions (96 hours). We used of the insecticide 6.6 mg L⁻¹ , 10 mg L⁻¹ and 20 mg L⁻¹ representing 30, 20, 10 times dilution, and of the fungicide – 30 mg L⁻¹, 38 mg L⁻¹ and 50 mg L⁻¹ representing 50, 40, 30 times dilution, respectively. These concentrations were considered as real applicable pesticide concentrations in plant protection practices. We can conclude that the tested fungicide showed more severe negative effects on gill histological structure of bighead carp compared to the tested insecticide. The tested fungicide showed a higher degree of negative impact on the occurrence of degenerative changes, associated with necrotic processes, as well as changes in the circulatory system, including vasodilatation and aneurysms. In contrast, the tested insecticide also caused degenerative changes, but to a lesser degree. Moreover, the insecticide toxicity is found to be connected more strongly with the proliferative alterations, indicating a different degree of expression. Hence, proliferation of the epithelial tissue indicates the

activation of compensatory adaptive mechanisms in the studied organ. On the other hand, the high degree of degenerative changes induced by the fungicide, affected the gill histological structure by thinning of the filaments and secondary lamellae, which could also affect the faster penetration of the toxicant through the gills. In addition, we found the highest degree of aneurysms after fungicide exposure, which is an indicator of the blood flow and a higher number of red blood cells, filling the vessels to compensate for the organ structural disturbances. Lastly, we consider that these results could be carefully taken into account in monitoring and risk assessment programs and when updating the legislation in the field of water conservation, as the studied pesticides are not yet considered as priority substances in surface waters according to EU legislation.

G7_14. Gecheva G., V. Yancheva, I. Velcheva, E. Georgieva, S. Stoyanova, D. Arnaudova, V. Stefanova, D. Georgieva, V. Genina, B. Todorova, I. Mollov. 2020. Integrated monitoring with moss-bag and mussel transplants in reservoirs. Water, 12: 1800.

ABSTRACT. In the present study, transplants were applied for the first time along with bags of moss and clams to study water quality in stagnant ponds. The tested species: *Fontinalis antipyretica* Hedw. and *Sinanodonta woodiana* (Lea, 1834) were collected from unpolluted sites and analyzed to obtain background levels. Then, the moss and mussels were left in cages for a period of 30 days in three reservoirs where both are not present naturally. Two of the reservoirs, Kardzhali and Studen Kladenets, suffer from old industrial pollution, and the third, Zhrebchevo reservoir, is affected by untreated waste. Twenty-four compounds were studied, among them trace elements Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn and organic priority substances: six polybrominated diphenyl ethers (PBDEs) congeners and short-chain chlorinated paraffins (SCCPs). The trace element accumulation was significant after the exposition period in all studied stations. PBDEs and SCCPs were also accumulated up to two times more in the moss tissues. PBDEs in the mussels exceeded the environmental quality standard (EQS). The applied combined transplants, and especially the moss-bags, revealed severe contamination with heavy metals not detected by the water samples. The moss and the mussel followed a different model of trace element and PBDEs accumulation. The SCCPs levels were alarmingly high in all plant samples. The study confirmed PBDEs and SCCPs as bioaccumulative compounds and suggested that an EQS for SCCPs in biota needs to be established.

G7_15. Stoyanova S., E. Georgieva*, I. Velcheva, I. Iliev, T. Vasileva, V. Bivolarski, S. Tomov, K. Nyeste, L. Antal, V. Yancheva. 2020. Multi-biomarker assessment in common carp (*Cyprinus carpio*, Linnaeus 1758) liver after acute chlorpyrifos exposure. Water, 12: 1837.

ABSTRACT. The excessive use of pesticides at different stages of crop production can pose a great danger to the aquatic environment, and particularly to fish. The purpose of the present work was to assess the negative effects of chlorpyrifos

(CPF) on the liver histological architecture and the activities of marker enzymes in common carp (*Cyprinus carpio* Linnaeus, 1758), by applying a multi-biomarker technique. Chlorpyrifos, O,O-diethyl O-3,5,6-trichloro-2-pyridyl-phosphorothioate) is a broad-spectrum organophosphorus insecticide (OP) used to control agricultural and domestic pests. In addition, CPF is one of the most widely used agricultural insecticides worldwide, accounting for 50% of global insecticide use. The tested insecticide is categorized as a priority pollutant in surface waters in terms of Directive 2013/39/EU. The carps were exposed to different and environmentally relevant CPF concentrations for 72 h (a short-term acute experiment). The results showed that the tested insecticide alters the liver histological structure, causing degenerative lesions, such as granular and vacuolar degeneration; necrobiotic alterations and necrosis, as well as changes in the circulatory system. In addition, CPF induces changes in the enzymatic activity of lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), cholinesterase (ChE), glutathione peroxidase (GPx) and catalase (CAT). The results from such experimental set ups could be successfully used in the legislation related to the protection of water bodies from contamination, in areas with intensive application of plant protection products used in agricultural practices, and also in implementing the Water Frame Directive by using multi-biomarker approaches.

G7_16. Yancheva V., E. Georgieva*, S. Stoyanova, I. Velcheva, D. Somogyi, K. Nyeste, A. Laszlo. 2019. A histopathological study on the Caucasian dwarf goby from an anthropogenically loaded site in Hungary using multiple tissues analyses. Acta Zoologica, 101(4): 431-446.

ABSTRACT. The present study aimed to investigate for the first time the health status of the Caucasian dwarf goby *Knipowitschia caucasica* (Berg, 1916, Fishes of freshwaters of Russian Empire, p. 563, Moscow, Russia: Dep. Zemledeliya) (Teleostei: Gobiidae) from an anthropogenically loaded site in Hungary using histopathological analyses on multiple tissues. For that purpose, fish were collected from the public beach at Tiszafüred near the River Tisza. Gills, liver and kidney were subjected to histopathological analyses, and the results showed different alterations in each organ, which also differed in their extent and severity. In addition, we also found lesions in the reproductive organs of both, male and female fish which, overall, we hypothesized could be due to untreated municipal wastewaters, most likely contaminated with endocrine-disrupting chemicals. The multi-organ histopathological analyses of Caucasian dwarf gobies revealed different lesions, prevalence and severity in each target organ, as follows: liver>gills>kidney>gonad (testes and ovaries). These histopathological lesions can be assessed as good indicators of contamination by endocrine-disrupting chemicals of freshwater ecosystems.

G7_17. Stoyanova S., I. Mollov, I Velcheva, E. Georgieva*, V. Yancheva. 2020. Cadmium and polyaromatic hydrocarbons exposure changes

the condition indices in *Dreissena polymorpha* (Pallas, 1771): A case study. Acta Zoologica Bulgarica, Supplement 15: 141-146.

ABSTRACT. Cadmium (Cd) and polyaromatic hydrocarbons (PAHs) are priority pollutants in surface waters according to Directive 2013/39/EC. They are toxic, persistent and tend to accumulate in high concentrations in aquatic organisms. In the present study, zebra mussel *Dreissena polymorpha* was confirmed as a bioindicator for contaminated freshwater ecosystems. We aimed to study the effects of short-term (96 h) and long-term (31 days) exposure to Cd and PAHs on the Condition index (CI) and Soft Tissue Wet Ratio (STWR) in zebra mussel. Overall, we found alterations in both CI and STWR (as compared to the control group) at all tested concentrations, including the one below the allowable concentration according to the EU legislation. These results demonstrated the toxicity of both contaminants. Furthermore, we confirmed that the studied condition indices could be successfully applied as biomarkers as they are fast and low-cost in future monitoring and risk assessment of polluted freshwater ecosystems.

G7_20. Georgieva E.*, V. Yancheva, S. Stoyanova, I. Velcheva, I. Iliev, T. Vasileva, V. Bivolarski, E. Petkova, B. László, K. Nyeste, L. Antal. 2021. Which Is more toxic? Evaluation of the short-term toxic effects of chlorpyrifos and cypermethrin on selected biomarkers in common carp (*Cyprinus carpio*, Linnaeus 1758). Toxics, 9(6): 125.

ABSTRACT. The general aim of this study was to investigate the negative short-term effects of different concentrations of chlorpyrifos (CPF) and cypermethrin (CYP), based on the EU legislation (MACEQS) in common carp (*Cyprinus carpio* Linnaeus, 1758) under laboratory conditions and to compare their toxicity. The fish were exposed to the pesticides for 96 h and then different histological and biochemical biomarkers were investigated in the gills and liver, and bioaccumulation analyses were conducted. The chemical studies showed increased pesticide concentrations in the gills as the first site for pollutants compared to the liver at the 96th hour. In addition, the histological analyses showed severe alterations in the gills and liver after exposure to both tested pesticides. In the gills, we found mainly intense proliferative and, to a lesser extent, degenerative changes and alterations in the circulatory system, such as necrosis and vasodilation. In the liver, regressive and progressive lesions, as well as circulatory disturbances and inflammation, were observed. The regressive lesions showed a higher degree of expression compared to the other changes. Furthermore, we found altered enzymatic activities—catalase, glutathione reductase, and glutathione peroxidase—in the liver, compared to the control. Overall, both tested pesticides impacted the studied biomarkers in common carp, even at concentrations lower than those permitted by law. However, the results of the comparative analysis showed a relatively higher toxicity of CYP compared to CPF in the fish. Still, questions persist as to whether the observed changes are adaptive or entirely destructive. To avoid any danger or risk, these pesticides must be applied cautiously, especially near water bodies.

Г7_19. Lambova S., T. Batsalova, D. Moten, S. Stoyanova, E. Georgieva, L. Belenska-Todorova, D. Kolchakova, B. Dzhambazov. 2021. Serum leptin and resistin levels in knee osteoarthritis—clinical and radiologic links: Towards precise definition of metabolic type knee osteoarthritis. Biomedicines, 9(8): 1019.

ABSTRACT. Obesity is considered a major risk factor for the development and progression of knee osteoarthritis (OA). Apart from the mechanical effect of obesity via increase in mechanical overload of weight-bearing joints, an association with hand OA has been observed. There has been increasing interest in the role of adipokines in the pathogenesis of OA in the recent years. It has been suggested that their systemic effects link obesity and OA. In this regard, the aim of the current study was measurement and analysis of serum levels of leptin and resistin in patients with knee OA with different body mass index (BMI). Seventy-three patients with primary symptomatic knee OA at the age between 35 and 87 years (mean age 66 years) were included in the study (67 women and 6 men). The patients were from 2nd to 4th radiographic stage according to Kellgren–Lawrence scale. 43 patients were with concomitant obesity (BMI ≥ 30 kg/m², mean values 38.34 ± 8.20) and 30 patients with BMI < 30 kg/m² (mean values 25.07 ± 2.95). Eleven individuals with different BMIs, including cases with obesity but without radiographic knee OA, were examined as a control group. Serum levels of leptin and resistin were measured via ELISA method. In patients with knee OA and BMI ≥ 30 kg/m², serum levels of leptin (39.546 ± 12.918 ng/mL) were significantly higher as compared with healthy individuals (15.832 ± 16.531 ng/mL, $p < 0.05$) and the patients with low BMI ($p < 0.05$). In patients with BMI < 30 kg/m² the levels of leptin (13.010 ± 10.94 ng/mL) did not differ significantly from the respective values in the control group ($p = 0.48$). Serum levels of resistin were also higher in knee OA patients in comparison with healthy controls, but the difference was statistically significant only for patients with high BMI (2.452 ± 1.002 ng/mL in the group with BMI ≥ 30 kg/m²; 2.401 ± 1.441 ng/mL in patients with BMI < 30 kg/m²; 1.610 ± 1.001 ng/mL in the control group, $p < 0.05$). A correlation was found between the serum levels of leptin and radiographic stage of OA, i.e., higher leptin levels were present in the more advanced 3rd and 4th radiographic stage, while for resistin a correlation was observed in the patient subgroup with BMI < 30 kg/m². Serum leptin and resistin levels and clinical characteristics were analyzed in patients with different clinical forms of OA. Novel clinical correlations have been found in the current study in patients with isolated knee OA vs. cases with presence of other disease localizations. It has been observed that patients with isolated knee OA were significantly younger and had higher BMI as compared with cases in whom OA is combined with other localizations i.e., spondyloarthritis \pm presence of hip OA and with generalized OA. This supports the hypothesis that presence of obesity promotes earlier development of knee OA as an isolated localization of the disease in younger patients before appearance of osteoarthritic changes at other sites. The levels of leptin and resistin in isolated knee OA were also higher. Serum levels of leptin and resistin in combination with patients'

clinical characteristics suggest existence of different clinical and laboratory profile through which more precise definition of metabolic phenotype of knee OA would be possible. Considering the fact that obesity is a modifiable risk factor that has an impact on progression of knee OA, different approaches to influence obesity may offer potential for future disease-modifying therapeutic interventions.

G7_20. Belenska-Todorova L., S. Lambova, S. Stoyanova, E. Georgieva, T. Batsalova, D. Moten, D. Kolchakova, B. Dzhambazov. 2021. Disease-modifying potential of metformin and alendronate in an experimental mouse model of osteoarthritis. Biomedicines, 9(8): 1017.

ABSTRACT. Osteoarthritis (OA) is the most common degenerative joint disease causing progressive damages of the cartilage and subchondral bone, synovial inflammation, and severe pain. Despite the complex pathomorphological changes that occur in OA, the approach to different forms of OA is standardized. The global results from pharmacological treatment are not satisfactory. Hence, this study aimed to explore the effects of metformin, alendronate, and their combination on OA development and progression in mice with collagenase-induced osteoarthritis (CIOA). Female ICR (CD-2) mice were randomized to five groups: control group, CIOA untreated, CIOA + metformin, CIOA + alendronate, and CIOA + metformin + alendronate. OA was induced by the intra-articular (i.a.) injection of collagenase. OA phenotype was analyzed by flow cytometry (bone marrow cell differentiation), ELISA (serum levels of the adipokines leptin and resistin), and histology (pathological changes of the knee joint). Treatment with metformin, alendronate, or their combination inhibited the expression of RANK and RANKL on osteoblasts and osteoclasts obtained by ex vivo cultivation of bone marrow cells in mineralization or osteoclastogenic media. In addition, metformin treatment was effective for the attenuation of fibroblast differentiation, but not of mesenchymal stem cells (MSCs), while alendronate had an opposite effect. The combination of metformin and alendronate had a suppressive effect on both MSCs and fibroblasts differentiation. Treatment with metformin, alendronate, and their combination decreased serum concentrations of leptin and resistin in the chronic phase of arthritis. The histopathological examination showed that compared with the untreated CIOA group (OA score 9), the groups treated with metformin (OA score 4) or alendronate (OA score 6) had lower scores for cartilage changes. Metformin combined with alendronate significantly decreased the degree of cartilage degeneration (OA score 2), suggesting that this combination might be a useful approach for the treatment of OA patients.

G7_21. Georgieva E.*, L. Antal, St. Stoyanova, D. Aranudova, I. Velcheva, I. Iliev, T. Vasileva, V. Bivolarski, V. Mitkovska, T. Chassovnikarova, B. Todorova, I.E. Uzochukwu, K. Nyeste, V. Yancheva. 2022. Biomarkers for pollution in caged mussels from three reservoirs in Bulgaria: A pilot study. Heliyon, 8(3): e09069.

ABSTRACT. The mussel-watch concept was firstly proposed in 1975, which was later adopted by several international monitoring programs worldwide. However, for the very first time, a field experiment with caged mussels was performed in three reservoirs in Bulgaria to follow the harmful effects of sub-chronic pollution (30 days) of metals, trace, and macro-elements, as well as some organic toxicants, such as polybrominated diphenyl ethers and chlorinated paraffins. Therefore, we studied the biometric indices, histochemical lesions in the gills, biochemical changes in the digestive glands (antioxidant defense enzymes, such as catalase, glutathione reductase, and glutathione peroxidase; metabolic enzymes, such as lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase, and the neurotransmitter cholinesterase), in addition to the DNA damage in the Chinese pond mussel, *Sinanodonta woodiana* (Lea, 1834) in Kardzhali, Studen Kladenets and Zhrebchevo reservoirs in Bulgaria. Significant correlation trends between the pollution levels, which we reported before, and the biomarker responses were established in the current paper. Overall, we found that both tested organs were susceptible to pollution-induced oxidative stress. The different alterations in the selected biomarkers in the caged mussels compared to the reference group were linked to the different kinds and levels of water pollution in the reservoirs, and also to the simultaneously conducted bioaccumulation studies.

G7_22. Yancheva V., E. Georgieva, I. Velcheva, I. Iliev, S. Stoyanova, T. Vasileva, V. Bivolarski, D. Todorova-Bambaldokova, N. Zulkipli, L. Antal, K. Nyeste. 2022. Assessment of the exposure of two pesticides on common carp (*Cyprinus carpio* Linnaeus, 1758): Are the prolonged biomarker responses adaptive or destructive? Comparative Biochemistry and Physiology, Part C 261: 109446.

ABSTRACT. Chlorpyrifos (CPF) and cypermethrin (CYP) are two insecticides that have a proven negative effect on non-target aquatic organisms when they enter the surface waters. However, literature on the comparative effects of these pesticides on important aquaculture fish species, such as common carp (*Cyprinus carpio* Linnaeus, 1758) is not yet scientifically detailed, especially over the long-term. The idea of conducting a long-term exposure is to find out how the observed biomarkers would change compared to the short-term exposure. In the natural environment, toxicants are not present alone, but in combination. By monitoring the long-term impact of individual substances, the state of aquatic ecosystems exposed to various toxicants could be predicted. Thus, this study aimed to evaluate the toxicity of different concentrations of CYP (0.0002, 0.0003, and 0.0006 µg/L) and CPF (0.03, 0.05, and 0.10 µg/L) in 50-L glass tanks on *C. carpio*, exposed for 30 days under laboratory conditions. A set of histological and biochemical biomarkers in the gills and liver were applied with the chemical analyses of water and fish organs. Furthermore, the condition and hepatosomatic index were calculated to assess the physiological status of the treated carps. The behavioral responses were also monitored, and the respiration rate was analyzed. The results suggest that CYP had a more prominent effect on the

histological structure of fish organs, biochemical responses of anti-oxidant enzymes, behavior, and respiration rate compared to the effect of CPF. In addition, the results also indicate that the liver is more susceptible to chronic and chemically induced cellular stress compared to the gills, with overall destructive changes in the histological biomarkers rather than adaptive. Regardless of the scenario, our results provide novel insights into pesticide exposure and the possible biological impacts on economically important freshwater fish, exposed to lower CYP and CPF concentrations, based on the EU legislation (maximum allowable concentrations, MAC-EQS).

G7_23. Kovacheva E., E. Georgieva, I. Velcheva, I. Iliev, T. Vasileva, V. Bivolarski, M. Nikolova, B. Todorova, D. Todorova-Bambaldokova, V. Yancheva, S. Tomov, S. Stoyanova. 2022. Histochemical and biochemical changes in common carp (*Cyprinus carpio* Linnaeus, 1785) liver after cypermethrin and chlorpyrifos exposure. *Ecologia Balkanica*, 14(2): 123-141.

ABSTRACT. Nowadays pollution of aquatic ecosystems with pesticides causes acute and chronic poisoning of fish, leading to serious damage to vital organs, such as the liver. Common carp (*Cyprinus carpio* Linnaeus, 1758) is a popular edible fish favored for culture due to its rapid growth, hardiness and reproduction in confined waters. The purpose of the present study was to investigate the negative effects of cypermethrin (CYP) and chlorpyrifos (CPF), based on their maximum allowable concentrations (Directive 2013/39/EU) on histochemical and biochemical biomarkers in the liver of common carp. The histochemical analysis included Periodic acid-Schiff staining (PAS reaction) and Sudan Black B staining, while in the biochemical study different hepatic enzyme activities such as lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT). The negative effects of the tested pesticides on fish were expressed with liver changes in the amount of glycogen and lipids, and enzyme changes of LDH, ASAT and ALAT, caused by the acute and chronic exposure to cypermethrin and chlorpyrifos under laboratory conditions. The results from such experimental set ups could be used in the legislation of protection water bodies from contamination, in areas near intensive application of plant protection products and also in implementing the Directive 2013/39/EU and Water Frame Directive by using multi-biomarker approaches.

G7_24. Kovacheva E., E. Georgieva*, I. Velcheva, M. Nikolova, P. Atanassova P., B. Todorova, D. Todorova-Bambaldokova, V. Yancheva, S. Stoyanova, S. Tomov. 2022. Acute histopathological changes in common carp (*Cyprinus carpio* Linnaeus, 1785) gills: pirimiphos-methyl, 2, 4 - dichlorophenoxyacetic acid and propamocarb hydrochloride effects. *Ecologia Balkanica*, 14(2): 143-159.

ABSTRACT. A number of characteristics make fish excellent experimental models in toxicological research, especially for the contamination of aquatic systems.

The main aim of the present study was to investigate the negative effects of different classes of pesticides (insecticide, herbicide and fungicide), based on their LC50 on the gills histological architecture of common carp (*Cyprinus carpio* Linnaeus, 1758). The effects of the tested pesticides on fish gills were expressed with histopathological alterations, such as proliferative, degenerative and changes in the circulatory system. Based on our results, the test insecticide showed higher toxicity with more severe irreversible necrotic changes in common carp gills compared to the herbicide and fungicide exposure. The identified histopathological changes in the fish gills can be successfully applied as reliable biomarkers for monitoring the degree of negative effects on the organisms due to the pesticide toxicity. The results from such experiments could be applied in the legislation in order to protect the water bodies from pesticide contamination, in areas with intensive application of plant protection products used in agricultural practices.

**Annotation of Published book chapter or collective monograph of assoc. prof
Elenka Stoilova Georgieva, PhD to criteria G8**

in accordance with the minimum national requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria.

G8_25. Yancheva V., S. Petrova, I. Velcheva, E. Georgieva. 2011. Ecological status of Topolnitsa River catchment area and Topolnitsa Dam. Proceeding of the 50th Anniversary Conference “Biological Sciences for Better Future”, University of Plovdiv “Paisii Hilendarski” Press, 267-280. ISBN 1312-062X.

ABSTRACT. The region (air, soils and waters) where the Topolnitsa River and the Topolnitsa Dam are located has been contaminated for many years. The main sources of contamination are the copper mines, metallurgy plants, non-ferrous smelters and mine tailings that have been left after the metals of interest such as: lead, zinc, copper, silver, gold and others have been extracted from the mineral rocks that contained them. The water of the river and the dam, which has been polluted for several decades with heavy metals, is used for drinking, watering agricultural lands and fishing. However, the data on the levels of pollution of the environment and the effects of these metals is very old or limited. Therefore, it is crucial that a full investigation and monitoring programs are carried out.

**Annotation of the scientific works of assoc. prof Elenka Stoilova Georgieva,
PhD outside of those indicated under criteria G7**

in accordance with the minimum national requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria.

26. Georgieva E.*, Sarafian V. 2012, Expression of blood group antigens A and B in pancreas of vertebrates. Journal of BioScience and Biotechnology, 1(1): 21-25.

ABSTRACT. The biological role of blood group antigens (BGA) A and B in tissues of different vertebrates is still controversial. There are few investigations on vertebrate pancreas and no obvious explanation of their tissue expression. The aim of the present study is to follow and compare the pancreatic expression of BGA A and B in representatives of five vertebrate classes. The biotin-streptavidin-proxidase labeling system was used for immunohistochemical detection of BGA by monoclonal antibodies to human A and B antigens. The present study reveals specific immunoreactivity in acinar and epithelial cells of pancreatic efferent ducts in species free-living vertebrates. The immunoperoxidase staining shows antigenic heterogeneity in the cellular localization. The number of positive cells and the intensity of expression vary in different species. Endothelial cells are positive only in the pancreas of *Emys orbicularis*. The lack of BGA A and B in some species suggests that the expression of these antigens is dependent not only on the evolutionary level of the species, but mainly on some genetic control mechanisms. The production of BGA A and B and the variability in their cellular localization probably reflect the stage of cell differentiation and the mechanisms of pancreatic secretor function. The presence of histo BGA in endodermal acinar pancreatic cells confirms the assumption for the high antigenic stability and conservatism of these molecules in vertebrate histogenesis and evolution.

27. Stoyanova S., E. Georgieva*, I. Velcheva, V. Yancheva, P. Atanasova. 2012. Effects of the insecticide "Actara 25 WG" on the glyconeogenesis in the liver of common carp (*Cyprinus carpio* L.). Journal of BioScience and Biotechnology, 1(3): 249-254.

ABSTRACT. The main goal of the present work is to study the effects of the new neonicotinoid insecticide „Actara 25 WG“ on the intensity of expression of glycogen in the liver of common carp (*Cyprinus carpio* L.) by using PAS-reaction on cryosections. Common carp is an economically important fish species, which is widely used as a bioindicator for the health of freshwater basins since it could also survive at very contaminated sites. We have used 6.6 mg/L, 10 mg/L and 20 mg/L of the test chemical under laboratory conditions. The results demonstrated that the intensity of staining of the PAS-reaction is directly proportional to the increasing concentration of the

insecticide. In addition, this indicates that the amount of glycogen in hepatocytes also increased. Conglomerates of accumulated glycogen in certain hepatocytes were found at the highest concentration of the insecticide. Therefore, we consider that under the influence of „Actara 25 WG“ the process of glyconeogenesis in the liver of the studied fish accelerates.

28. Georgieva E.*, P. Atanasova, I. Velcheva, S. Stoyanova, V. Yancheva. 2013. Histochemical effects of “Verita WG” on glycogen and lipid storage in Common carp (*Cyprinus carpio* L.) liver. *Ecologia Balkanica*, 5 (2): 91-97.

ABSTRACT. The research is based on an important and current problem concerning the anthropogenic pollution of water bodies in the environment with pesticides, the content of which threatens the health of aquatic organisms, bones and humans. A resistant, widespread and economically important species of common carp (*Cyprinus carpio*, L.) is used as the object of research. We aimed in the present work is to study the effects of fosetyl-Al and fenamidone based fungicide (“Verita WG”) on glycogen storage and expression of lipid droplets in common carp (*Cyprinus carpio*, L.) liver. Concentrations of the test chemical were 30 mg/L, 38 mg/L and 50 mg/L under laboratory conditions. We used PAS-reaction for detection of glycogen storage and Sudan III staining for detection of lipid droplets in common carp hepatocytes. Hence, we found that the amount of glycogen and the fat storage in the liver increased proportionally with the increased fungicide concentrations. We also found conglomerates of accumulated glycogen in certain hepatocytes at all used concentrations. Overall, the results demonstrated enhanced glyconeogenesis and fat accumulation in the common carp liver, exposed to the test chemical.

29. Velcheva I., E. Georgieva*, P. Atanassova. 2013. Gill tissue recovery after copper exposure in *Carassius gibelio* (*Pisces: Cyprinidae*). *Central European Journal of Biology*, 8(11): 1112-1118

ABSTRACT: We investigated the influence of copper in a long-time treatment with concentrations of 0.05 mg L⁻¹ and 0.1 mg L⁻¹ to track the histopathological changes in gills of *Carassius gibelio*, and to find at what extent they will recover after the effect of the copper concentrations stops. Treatment with copper lasted 21 days and the recovery time was of the same duration. The results of histological examination showed degenerative changes (resulting in thinner secondary lamellae and filamentary epithelium), and hyperplastic and hypertrophic changes (proliferation, vasodilatation, aneurysms, epithelial interstitial edema, and fusion) in gills under the influence of two concentrations. The degenerative changes have higher prevalence at low concentrations, while hyperplastic and hypertrophic ones – at high concentrations. After the period of recovery they remained the same, but the extent of expression on the surface of gill filaments changed. The long-time copper intoxication in low concentrations of copper affects gill structure, causing severe changes whose recovery is a slow process that requires a longer period of time.

- 30. Stoyanova S., V. Yancheva, I. Velcheva, P. Atanasova, E. Georgieva*. 2015. Thiamethoxam causes histochemical changes in the liver of *Aristichthys nobilis* Rich., 1845. Journal of BioScience and Biotechnology, 4(3): 321-325.**

ABSTRACT. In the present study, we aimed to investigate the effects of the neonicotinoid insecticide thiamethoxam on the hepatic glycogen in bighead carp (*Aristichthys. nobilis* Rich.). Fish were exposed to 6.6 mg/L, 10 mg/L and 20 mg/L of the insecticide under laboratory conditions for 96 hours. The PAS-reaction was applied to liver cryostat sections in order to indicate the amount of glycogen. The results showed that the hepatic glycogen amount increased with increasing the insecticide concentrations. On the other hand, we observed glycogen conglomerates in certain hepatocytes. Hence, our results demonstrated an enhanced process of glycconeogenesis in the fish liver under the influence of thiamethoxam.

- 31. Todorova K., I. Velcheva, V. Yancheva, S. Stoyanova, S. Petrova, E. Georgieva. 2015, Effects of nickel and its combination with other heavy metals (Cd, Pb, Zn) on common carp (*Cyprinus carpio* Linnaeus, 1785). Trakia Journal of Sciences, 13, Supplement 2: 324-328.**

ABSTRACT. We aimed to study ex-situ the effects of acute toxicity test with Ni²⁺, Ni+Cd, Ni+Pb and Ni+Zn on behavior, survival and oxygen consumption of common carp, *Cyprinus carpio* L. Behavioral effects were more pronounced in fish exposed to 0.2 mg/l Ni²⁺, 0.3 mg/l Ni²⁺ and 0.45 mg/l Ni²⁺. Common stress reactions, such as anxiety, jumps and quick movements, and also accelerated movements of the fish gill covers were observed. Fish behavior from the lower Ni²⁺ concentrations (0.05 and 0.1 mg/l Ni²⁺) and from the test combinations of Ni+Cd, Ni+Pb and Ni+Zn was quite different. In general, during the first hours of the experiment the fish were anxious, but after 48 hours they started to be lethargic, which was expressed in their slow movements. Fish survival was 90% under intoxication with 0.2 mg/l Ni²⁺ and combination of Ni+Pb. In the rest of the tested heavy metal concentrations it was 100%. Data on the respiration intensity rate and oxygen consumption of fish exposed to 0.2 mg/l Ni²⁺, 0.3 mg/l Ni²⁺, Ni+Pb and Ni+Cd were lower in comparison with the control group. This result indicated that the Ni²⁺ ions impacted the fish respiratory system. Depending on their toxic effect the descending row of the studied heavy metals could be presented as follows: Ni²⁺ > Ni+Pb > Ni+Cd > Ni+Zn. Overall, such experiments could be successfully applied in environmental monitoring and risk assessment programs for metalcontaminated aquatic ecosystems and toxic effects on fish.

- 32. Yancheva V., I. Mollov, I. Velcheva, E. Georgieva, S. Stoyanova. 2016. Effects of cadmium (Cd) on the lysosomal membrane stability and respiration rate of two freshwater mollusks under ex situ exposure: preliminary data. South Western Journal of Horticulture Biology and Environment, 7(1): 27-34. (indexed in Scopus)**

ABSTRACT. Environmental pollution by metals, among other chemicals, involves major health risks to all living organisms, humans and wildlife. Heavy metals are toxic, non-biodegradable and persistent environmental pollutants. One of the most common pollutants is Cd, which is mainly used in the production of stabilizers and pigments in plastics, in the electroplating industry and is also released as a by-product from other anthropogenic activities, including mining, metallurgy and agriculture. Cd, which is considered a priority toxic substance in surface water according to Directive 2008/105/EO (2008). For monitoring the health of coastal systems, sentinel organisms such as mussels have been identified as suitable candidates for indicating contaminant levels in the aquatic environment and as such have been proposed as suitable 'biomonitors' of pollution. The research is based on the development of the research objective related to the presentation of some preliminary data on the effects of Cd on lysosomal membrane stability and respiration rate in two invasive and environmentally resistant freshwater molluscs, the Chinese marsh mussel (*Synanodonta woodiana*) and zebra mussel (*Dreissena polymorpha*). The study was conducted in laboratory conditions with exposure for 72 hours. In the Cd-treated mussel species tested, a significant decrease in lysosomal destabilization indices with lower retention time and an increase in respiratory rate index was observed compared to the control. In general, the tested species were found to be sensitive to Cd exposure in terms of the two studied biomarkers – lysosomal membrane stability and respiration rate.

33. Yancheva V., I. Mollov, I. Velcheva, S. Stoyanova, E. Georgieva. 2016. Cadmium (Cd) affects the gill structure and respiration rate of common carp (*Cyprinus carpio* L.). ZooNotes, 97: 1-4.

ABSTRACT. The main purpose of the present study was to provide some preliminary data on the effects of Cd, which is considered as priority toxic substance in surface waters according to Directive 2008/105/EO on the gill structure and respiration rate of common carp (*Cyprinus carpio* L.) under ex situ conditions. We observed significant histological changes, which were grouped as proliferative and degenerative ones, as well as increase in the respiration rate index in the treated with Cd fish, compared with the control. In general, the tested fish species proved to be sensitive to Cd exposure in terms of the studied parameters.

34. Stoyanova S., E. Georgieva* I. Velcheva, P. Atanasova, V. Yancheva. 2019. Lipid accumulation in *Cyprinus carpio* (Linnaeus, 1785) liver induced by thiamethoxam. ZooNotes, 139: 1-4.

ABSTRACT. The aim of the present study is to investigate the effects of a thiamethoxam based insecticide on the expression of lipid droplets in Common Carp, *Cyprinus Carpio* (Linnaeus, 1785) liver. The selected concentrations of the test pesticide were 6.6 mg/L, 10 mg/L and 20 mg/L under laboratory conditions for an acute period of 96 h. The Sudan III staining method was applied for detection of fatty

degeneration in the fish hepatocytes. Overall, we found that the fat storage in the liver cells increased proportionally with the increased pesticide concentrations. The results demonstrated fat accumulation in the fish liver which in addition, could be used as an easy to perform and relatively inexpensive biological tool for studying the effects of pesticide contamination on fish.

- 35. Yancheva V., E. Georgieva*, I. Velcheva, P. Atanasova, S. Stoyanova. 2019. Histochemical alterations in liver of Common carp *Cyprinus carpio* (Linnaeus, 1785) after glyphosate exposure: Preliminary study. ZooNotes, 137: 1-4.**

ABSTRACT. In recent years, glyphosate has been the world's best-selling herbicide used in agricultural and non-agricultural areas, and its use for crop production is widespread worldwide, both in industrialized and developing countries. The present study was designed to provide some preliminary data on the toxic effects of 96 h exposure to glyphosate on the liver of Common Carp (*Cyprinus carpio* L.) under ex situ conditions. For this purpose we used Sudan III staining which could be suggested as fast and low-cost histochemical biomarker for pesticide contamination effects. Overall, from the obtained results on histochemical alterations in the liver of Common Carp after glyphosate exposure changes we observed a tendency towards an increase of the lipid content in the hepatocytes along with the increase in the concentration of glyphosate.

- 36. Tomov S., R. Stoyanova, E. Georgieva, P. Atanassova, I. Dechev. 2019. Alterations of sperm chromatin integrity in sub/infertile men treated with nutritional supplement papa. Scientific works of the Union of Scientists in Bulgaria, Plovdiv, series G. Medicine, Pharmacy and Dental medicine, 22, 259-265.**

ABSTRACT. In recent years, dietary supplements have been used to treat male sub/infertility, which could improve the spermatogenesis and morphophysiological status of the sperm cells. Common causes of the male sub/infertility include anomalies in the spermatozoa chromatin. The present study aims to determine the effects of PAPA nutritional supplement on sperm chromatin integrity using toluidine blue test. A group of men (n = 68) with reproductive problems and a pronounced sub/infertility was studied, after a written agreement and a completed questionnaire, required by the current Bulgarian legislation. A test group of men (n = 58) with abnormalities in the semen analysis and diagnosis, took the food supplement for three months. Another group (n = 10) with normozoospermia did not take a dietary supplement and served as a control, respectively. The patients from the test group were divided into groups according to their age; unhealthy working environment (I-IV); harmful habits (smoking I-III) and diagnosis. The results showed an improvement in the values of the chromatin integrity that ranged in the test groups. The total percentage change after the treatment

was 9.58%. Essential to the effectiveness of the applied treatment with the nutritional supplement were the additional exogenous factors such as unhealthy work environment and habits.

- 37. Yancheva V., Stoyanova S., Velcheva I., Georgieva E. 2020. Fish as indicators for environmental monitoring and health risk assessment regarding aquatic contamination with pesticides. International Journal of Zoology and Animal Biology, 3 (1): 1-6.**

ABSTRACT. The proximity of water basins to anthropogenic sources of pollution affecting the state of nature also determines the need to study the ecosystems existing there. Fish are used as reliable indicators of pollution of the aquatic environment. Changes in the fish body make it possible to determine the toxicity of the contaminated water and the potential danger posed by anthropogenic substances that have entered it. In order to make a comprehensive assessment reflecting the effects of organic pollutants, such as pesticides in aquatic ecosystems, monitoring only one biomarker in fish is not sufficient enough. A complex assessment is required, including both tissue changes and changes in enzyme activity. It is also necessary to compare the effect of priority organic pollutants, both in the laboratory and in the field. Applied concentrations, exposure time, and species sensitivity play an important role in determining the extent of changes in the fish body. On the basis of a comparison of these factors, a model for assessing the pollution of aquatic ecosystems with priority organic pollutants included in Directive 2013/39/ EU can be built [23,24]. This model can be applied both, in agricultural practices and ecological monitoring in order to prepare an adequate regulatory framework, which includes assessment of changes in biota.

- 38. Yancheva V., I. Mollov, S. Stoyanova, B. Todorova, I. Velcheva, E. Georgieva. 2021. Toxic pesticides effects on the respiration rate in *Dreissena polymorpha* (Pallas, 1771). ZooNotes, 173: 1-4. (indexed in WebSci)**

ABSTRACT. With the present experiment we aimed to study the possible negative effects of two commonly used insecticides – cypermethrin (CYP) and chlorpyrifos (CPF) on the respiration rate of Zebra Mussel (*Dreissena polymorpha* Pallas, 1771) after 96 hours and 30 days. We found that both chemicals altered the respiration process; however CYP was determined to be more toxic regarding this particular biological measurement.

- 39. Georgieva E.*, I. Velcheva, S. Stoyanova, V. Yancheva, A. Vladikov. 2021. Ecological and economic considerations for water resources as water capitals /the case of fish toxicology in the “Vita plus” project. Knowledge – International Journal, 45(3): 549-554.**

ABSTRACT. This paper is focused on presenting some ecological and economic aspects related to reconsider water resources as water capitals, in the forthcoming concept of introducing bioeconomy in the new EU sets of strategies, policies, and regulations. Financing of this paper is provided by the “Vita Plus” project (2021-2022) – a transdisciplinary project, executed by researchers of the University of Plovdiv. The object of this paper is to conduct a research and demonstrate through the “Vita Plus” project complex methods of detecting toxicity in waters. Furthermore, the focus of the paper is to provide an adequate model for measuring toxicity levels of waters by conducting reliable toxicology research on fishes from polluted waters. We aimed to set a reliable laboratory practice, which may be utilized to deliver comparable indicators, as prescribed by EU policies and regulations in the forthcoming programming period 2021-2027, as there will be a general shift towards circular economy of no to minimum waste in nature.

40. Georgieva E.*, R. Stoyanova, V. Yancheva, I. Velcheva, S. Petrova, S. Stoyanova, S Tomov. 2022. Is there a correlation between impaired sperm quality and overweight /obesity?: A eview. Acta Morphologica et Anthropologica, 29(3-4): 120-127 (indexed in WebSci)

ABSTRACT. Obesity is associated with significant disturbance in the hormonal status that can affect the reproductive system. In recent decades, an increasing interest in related to the association between high BMI levels, obesity and decreased sperm quality, which could also lead to a decrease in male reproductive potential. The aim of the present work is to identify the basic mechanisms of impaired sperm quality due to overweight and obesity. Sedentary lifestyle and work, as well as age of men are defined as possible ways to elevated BMI levels. Both inflammation and oxidative stress (as related pathophysiological processes) are considered as basic mechanisms, which could be found in the pathogenesis of male infertility caused by high BMI levels and obesity.

41. Yancheva V., S. Stoyanova, B. Todorova, E. Georgieva, I. Velcheva. 2022. Ingestion of plastics in the European bass (*Dicentrarchus labrax* Linnaeus, 1758): first known observation in the city of Plovdiv, Bulgaria. Zoonotes, 191: 1-4. (indexed in WebSci)

ABSTRACT. This article reports the first recorded case of plastic items in a sea bass (*Dicentrarchus labrax* Linnaeus, 1758) purchased from a local fish market in the city of Plovdiv. This observation confirms the threat to the health of marine organisms from the increase of plastic in sea basins. The growing trend in the production and use of plastics is on a global scale, which inevitably causes them to end up as waste products in the marine environment. The annual introduction of plastic into the ocean is estimated in millions of tons. The multiple risks that plastics pose to marine life have prompted their inclusion in some international legislation and marine conservation

projects, such as the European Marine Strategy Framework Directive (MSFD) and the Marine Debris Program of the US National Oceanographic and Atmospheric Administration (NOAA).

- 42. Yancheva V., S. Stoyanova, B. Todorova, E. Georgieva, I. Velcheva. 2022. Zebra mussel (*Dreissena polymorpha* Pallas, 1771): the invasive bioindicator for freshwater quality? *Zoonotes*, 197: 1-4 (indexed in WebSci)**

ABSTRACT. In this short review we aim to discuss the advantages and disadvantages of using zebra mussel (*Dreissena polymorpha* Pallas, 1771) for the purposes of freshwater monitoring.

- 43. Yancheva V., S. Stoyanova, B. Todorova, E. Georgieva*, I. Velcheva. 2022. Common carp (*Cyprinus carpio* Linnaeus, 1785): a species equally important for aquaculture and aquatic toxicology. *Zoonotes*, 199: 1-3. (indexed in WebSci)**

ABSTRACT. In the presented brief review, the main objective is to present the main positive aspects of using common carp (*Cyprinus caprio* Linnaeus, 1785) for both aquaculture and aquatic toxicology purposes. Carp is also the most commonly farmed fish in aquaculture in Bulgaria, along with brown trout (*Salmo trutta fario*, Linnaeus, 1758) and rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792). Based on long-term experiments with carp, we believe that it is an excellent test organism and may be applied in ecotoxicological studies because it is relatively resistant to water pollution, which is essential for the selection of bioindicators in laboratory and field experiments.

- 44. Todorova B., D. Todorova-Bambaldokova, S. Stoyanova, E. Georgieva, I. Velcheva, V. Yancheva. 2023. Microplastic pollution – are there potential toxic threats for aquatic animals in Bulgaria? *Zoonotes*, 212: 1-4 (indexed in WebSci)**

ABSTRACT. Microplastic (MPs) pollution and its negative effects is a growing, yet poorly studied problem in Bulgaria. In the present review we aimed to summarize the available data on MPs contamination of surface waters and sediments, and its impact on aquatic animals in Bulgaria.

Annotation of textbooks and teaching aids of assoc prof. Dr. Elenka Stoilova Georgieva according to criteria E19 and E20

in accordance with the minimum national requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria.

45. Georgieva E. 2022, General Histology, University Publishing House "P. Hilendarski, Plovdiv, p. 259., ISBN 978-619-202-812-1

ABSTRACT. The General Histology textbook contains a total of 13 sections that include topics from the Histology curriculum. The textbook provides valuable information on the general principles in the structure, functions, and origin of the types of tissues that make up the body of multicellular animals and man, as well as partial elements of comparative and special histology. Varieties of the main tissue types are presented, with their morphological and physiological features. The information in the General Histology textbook supports students in mastering, assimilating and systematizing modern knowledge of the normal functional morphology of tissues, which will be useful for their understanding of related scientific disciplines such as anatomy, special histology, embryology, physiology, biochemistry, etc.

46. Georgieva E. 2022. General Pathology, University Publishing House "P. Hilendarski", p. 209. ISBN 978-619-202-813-8

ABSTRACT. The General Pathology Textbook is a short course in General Pathology that includes topics tailored to the students' curriculum. The textbook contains a total of 8 sections in which information is presented on the subject, tasks and methods of pathology, the main pathological changes in the cell and intercellular substance, disorders in blood circulation and lymph circulation, basic immunopathological and compensatory-restorative processes, necrosis, apoptosis, as well as basic tumor information. The proposed learning content provides in-depth knowledge of classical and well-established knowledge about pathomorphological changes for some basic diseases and processes, as well as some of the new scientific achievements in the field of general pathology.

47. Еленка Георгиева, 2022, Guide on General Embriology, University Publishing House "P. Hilendarski", p.106, ISBN 978-619-202-816-9

ABSTRACT. The guide to general embryology includes a brief introduction to embryology as a science and basic research methods. Brief historical notes on the development of embryology are also presented, as well as information on histological technique for making embryological preparations. Brief and summarized information is included on gametogenesis (spermatogenesis and oogenesis), types of male gametes,

types of female gametes and egg shells, embryonic development and stages of embryonic development - furrowing, gastrulation, organogenesis, as well as features of embryonic development in the class Birds (Aves) and subclass Placental mammals (Placentalia, Eutheria). For each topic, tasks are presented with detailed guidelines regarding their development and the necessary practical knowledge related to analyzing the preparations provided to the students. For practice and assessment of knowledge, a variety of test tasks are included for each topic.

Plovdiv,

12. 02. 2023

Signature:

(Assoc. Prof. Elenka Georgieva, PhD)