

**University of Life Sciences “King Mihai I”  
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**Doctoral School: Plant and Animal Resources Engineering (IRVA)**

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**NUTRITIONAL STRATEGIES IN HEAVY METAL DETOXIFICATION,  
ALTERNATIVE TO THE USE OF SYNTHETIC CHELATING AGENTS**

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## ABSTRACT

Habilitation thesis entitled **"Nutritional strategies in heavy metal detoxification, alternative to the use of synthetic chelating agents,,** is structured according to the legislation in force and the regulation of the "King Mihai I" University of Life Sciences of Timisoara, regarding the organization and development of the process of obtaining the habilitation certificate, in order to acquire the quality of Ph.D. coordinator.

The habilitation thesis is structured in two parts as follows:

PART I. OWN SCIENTIFIC, PROFESSIONAL AND ACADEMIC CONTRIBUTIONS

PART II. EVOLUTION AND CAREER DEVELOPMENT PLAN

The summary of the habilitation thesis is written in two versions, in Romanian and English, followed by the presentation of scientific, professional and academic achievements, on interdisciplinary thematic directions, indicating plans for the evolution and development of one's own professional, scientific and academic career, respectively research-teaching directions.

The scientific, professional and academic achievements obtained during the postdoctoral period (1999-2023) focused on the study of metabolic, biochemical and histopathological tissue changes induced by chronic heavy metal poisoning in freshwater fish species; testing of natural vegetal and algal sources rich in active principles with chelating and antioxidant properties, in chronic heavy metal poisoning at *Carrassius gibelio* Bloch, but there were also concerns in other areas specific to Domestic Animal Physiology and Human Physiology.

The scientific research carried out in the postdoctoral period and presented in this habilitation thesis can be systematized on the following interdisciplinary thematic directions of research, as follows:

Research on establishing reference intervals of blood values of biochemical parameters in freshwater fish species representative for our country;

- multidisciplinary approach to the distribution and highlighting of tissue toxicities of heavy metals (mercury, cadmium, lead) in *Carassius auratus gibelio* Bloch species, with a view to improving knowledge of the cellular and tissue mechanisms of their toxicity to fish;
- multidisciplinary approach to the potential benefits and risks of the use of synthetic chelating agents on mineral homeostasis and tissue and functional structural integrity in fish exposed to cadmium contamination;
- multidisciplinary approach on detoxifying capacity of natural plant and algal sources with chelating and antioxidant properties in experimental cadmium and lead contamination in freshwater fish species.

### **Cap. I. Research on establishing the reference intervals of blood values of biochemical parameters in freshwater fish species representative for our country**

Measurement of blood biochemical parameters is a non-lethal means of determining health status, and they are considered basic physiological markers in fish.

Physiologists shall use biochemical index reference values for the assessment of stress responses, nutritional status, reproductive status, tissue damage caused by handling procedures and health, exposure to contaminants, parasitic infestation, inadequate population density and agricultural

practices for the detection and diagnosis of metabolic disorders, disease response, assessment of pollution of the aquatic environment, aquatic ecosystems and their land.

The assessment of health status or diagnosis in warm-blooded animals is facilitated by the existence of an impressive database of normal haematological and biochemical values. However, at the time of starting our study, the lack of data expressing normal and pathological limits of blood biochemical parameters was the main reason why blood analysis was not part of the routine tests used to assess the health of poikilothermal animals of economic interest. As such, we intend to supplement the data from the specialized literature, with reference values that will later serve to establish seasonal variations, those related to age, living conditions, physiological state, etc.

**Activity. 1. Measurement of serum biochemical parameters in representative fish species belonging to the family Ciprinidae, Salmonidae, Esocidae and Acipenseridae.**

Among the fish species that were the subject of our study are: common carp (*Cyprinus carpio*), Romanian Danube carp, mirror carp (*Cyprinus carpio specularis*) of Szeged, Prussian carp (*Carassius gibelio* Bloch), bream (*Abramis brama*), pike (*Esox lucius*) - rainbow trout (*Oncorhynchus mykiss*), Siberian sturgeon (*Acipenser baerii*), sterlet (*Acipenser ruthenus*), Russian sturgeon (*Acipenser gueldenstaedtii*). The biological material came from both natural habitat and recirculating growth units.

The considered serum biochemical parameters (glucose, total protein, albumin, urea, uric acid, creatinine, triglycerides, cholesterol, GOT, GPT, GGT, alkaline phosphatase, alpha-amylase, bilirubin, Ca, P, Mg, Fe) registered significant differences between the 4 families compared - Ciprinidae, Esocidae, Salmonidae, Acipenseridae - which may be due to a large number of factors: interspecific variability, environmental conditions (natural environment or captivity, water temperature and chemistry, water depth, water source), season, physiological and nutritional status of test specimens.

Thus, the high level of serum calcium recorded in Ciprinidae can be attributed, in the case of females, to the specific breeding season, calcium being an indicator of the intensity with which vitelogenin synthesis takes place.

Unlike calcium, which fish can take up from water, phosphorus is considered the most critical macromineral in fish feed, and whose availability in water is limited, showed the highest values in Salmonidae, which can be explained by that, under intensive growing conditions, it is administered as a mineral supplement in different proportions.

Also in Salmonidae, another blood parameter - urea - produced by the liver and excreted by the gills, had increased values, without being incriminated as being due to starvation or disturbance of liver function.

The elevated serum cholesterol levels found in all species except Esocidae may be the result of mobilization of energy reserves during the pre-spawning period to support the increased requirements for precursors necessary for cell membrane reconstruction (phospholipids) and cholesterol as a substrate for steroidogenesis. Similarly, high serum triglycerides (in Acipenseridae and Salmonidae) may be due to increased metabolic rate, but also to additional food availability due to intensive operation.

Concluding, the biochemical analysis of blood parameters in the mentioned fish species reveals a great intra- and interspecific variability. Variations that can be influenced by numerous endogenous and exogenous factors. The values of serum metabolites and enzymes obtained in this study may serve as reference values for monitoring the metabolic balance and health status of fish species in natural aquatic biocenosis or those exploited in intensive rearing systems. In addition, reported values of serum biochemical parameters may be used as sensitive biomarkers in ecotoxicological studies on the

effects of different contaminants in the aquatic environment on the health status of aquatic organisms of economic concern.

**Cap. II. Multidisciplinary approach to the distribution and highlighting of tissue toxic valences of heavy metals in the *Carassius gibelio* Bloch species, in order to improve knowledge about the cellular and tissue mechanisms of their toxicity to fish**

World wide environmental protection is one of the most acute and complex issues of the present. The interrelations of the environment with the fields of economics and with all aspects of social life determine mutual conditioning. The impetuous economic and social development of human communities has induced accelerated environmental change, profoundly disrupting the natural balance of processes in the biosphere. Among different types of pollution, chemical pollution affects all components of the biosphere, implicitly the activity of aquatic ecosystems, radioactive, chemical or biological impurities endangering their balance. The presence of chemical pollutants in water can have particularly serious ecological consequences by restructuring biocenosis, altering their integrity and, implicitly, aquatic ecosystems.

Heavy metals and their toxic salts - which show great stability, are considered hazardous agents for aquatic animals. Some heavy metals are essential components of many respiratory enzymes and pigments, while Hg, Cd and Pb are non-essential and highly toxic, even at very low levels, especially if they accumulate in metabolically active tissues, potentially inducing mutations with serious consequences for future generations of aquatic life, with profound implications for ecosystem stability.

**Activity 1. Determination of the magnitude of tissue bioaccumulation of Hg, Cd and Pb in *Carassius gibelio* Bloch species, under the conditions of induction of chronic intoxication with mercuric chloride  $\text{HgCl}_2$ , cadmium acetate  $\text{Cd}(\text{CH}_3\text{COO})_2 \times 2\text{H}_2\text{O}$  and lead nitrate  $\text{Pb}(\text{NO}_3)_2$ .**

When choosing the test organisms, the criterion of accessibility and representativeness from an ecological point of view was taken into account, which is why we focused on the species *Carassius gibelio* Bloch from the Cyprinidae family, class Pisces. Besides the fact that this species is easy to acquire, the juvenile goldfish is large and easily adapts to captive conditions.

The 1-year-old juvenile, distributed in 3 experimental groups, was exposed to Hg, Cd and Pb induced poisoning. The investigated heavy metals - Hg, Cd and Pb respectively - were administered to aquarium water at concentrations of 0.25 ppm, 1.625 ppm and 75 ppm, respectively, the three doses being determined based on the mean lethal  $\text{LC}_{50}$  concentration for an interval of 3 weeks.

The spectrometry determinations performed at the end of the experimental period revealed that, regardless of the tissue considered, the exposure of goldfish specimens to sublethal doses of Hg, Cd and Pb resulted in an increase in their tissue level (bioaccumulation).

Thus, following the take-up from the water, Hg is distributed throughout the body of the fish and tends to concentrate in a tissue-specific manner, the magnitude of bioaccumulation being achieved in the following descending order: gills>liver>intestine and kidneys>muscle>skin>brain>heart. For silver carp specimens, Cd shows increased accumulation affinity for intestinal, renal, hepatic, and gill tissue, followed by the other tissues analyzed, in the order: intestine>kidney>liver>gills>skin>heart>brain>muscle. In chronic lead nitrate poisoning, Pb showed the following pattern of tissue bioaccumulation: liver>kidney>gills>intestine>brain>skin>muscle>heart.

The rapid bioaccumulation of heavy metals in the tissues of intoxicated specimens allows us to affirm that the rate of uptake exceeds the rate of their metabolism and excretion. Therefore, measuring the level of bioaccumulation of heavy metals in fish tissues is a useful method in biomonitoring the exposure of aquatic biocenoses to pollutants, fish can be true biomarkers for assessing the level of heavy metal contamination of the aquatic environment and the health of aquatic ecosystems.

**Activity 2. Description of tissue histopathological effects of heavy metal contamination in *Carassius gibelio* Bloch belonging to the family Cyprinidae**

In order to microscopically examine the tissue lesions induced by Hg, Pb and Cd, as well as the degree of impairment of their function, our research continued with obtaining the permanent histological preparation, for staining using the Mallory trichromic method.

Heavy metals taken in various ways from water showed cumulative effect in the tissues of goldfish specimens, the most affected being the gills, liver and kidney tissue. Once installed, chronic heavy metal poisoning induces serious histopathological lesions, which resulted in compromising the functions of the target organs targeted by that metal. The structural tissue alterations revealed by microscopic analysis have had different levels of severity, depending on the type of contaminant, implicitly on the dose used.

Thus, lesions reported at the level of gills: dystrophic processes by superficial cell detachments from the cell layer with extended internal epithelial cell layer (Hg), multilayered epithelium, hyperplastic processes, leukocyte infiltrate, in subepithelial connective tissue, vascular ectasias (Cd), disorganization of gill blades, vascular ectasias and hemorrhagic areas, leukocyte infiltrates (Pb), suggest that sublethal doses of the 3 contaminants affect the respiratory barrier, reducing the surface area for gas exchange, while also affecting ion exchange capacity.

Chronic exposure to Hg did not induce major histological changes in intestinal epithelium (mild dystrophic processes, leukocyte infiltration); instead, the large number of leukocyte infiltrative cells, the hypertrophic capillary network in the chorion structure (Cd), plus capillary fragility and the appearance of small bleeding areas (Pb), suggest that the intestinal mucosa is one of the access routes of heavy metals into the fish organismal.

At liver level, microscopic images incriminate a strong hepatotoxic effect of Hg, Cd and Pb, namely: fibrosis of peri- and interlobular connective tissue vascular ectasias, numerous lymphocyte infiltrates, vacuolar cytoplasm and pycnotic nucleus (Hg); hypertrophic blood vessels, with hemolyzed content, leukocyte infiltrates, vacuolar cytoplasm, atrophic hepatocytes, congestive processes, fragmented chromatin, until extinction (Cd); hypertrophic hepatocytes, vacuolized cytoplasm, karyorexis and karyolysis, congestive capillaries (Pb).

Morphological lesions caused by Hg, Cd and Pb extend to the kidney: thickening of the endothelial wall of renal corpuscles, macrophago-mesangial infiltrative cells (glomerulonephritis), nephrocytic hyperplasia, cytoplasmic vacuolizations, pycnotic nuclei (Hg); capillary ectasias with erythrocyte extravasation, uriniferous tubules with layered epithelium, presence of giant cells, dystrophic nephrocytic processes (cellular hypertrophies, pycnoses and karyorexis, cytoplasm and nucleus degeneration) (Cd); glomeruli with dense bleeding areas, atrophic nephrocytes, basophilic hyalinized areas, ectasias, vascular congestion and hemorrhagic lakes (Pb).

**Cap. III. Multidisciplinary approach to potential benefits and risks of using synthetic chelating agents on mineral homeostasis and tissue and functional structural integrity in fish exposed to cadmium contamination**

Heavy metals are considered harmful pollutants to aquatic organisms by themselves or by their toxic salts, which are highly stable. Cadmium is considered one of the most toxic environmental substances due to its ubiquity, toxicity and long half-life. Compared to other heavy metals, cadmium and its compounds are relatively soluble in water, are easy to mobilize, have a higher bioavailability and tend to accumulate in living tissues. In addition, cadmium interacts with other essential tissue elements, manifesting antagonistic effect towards them, inducing severe biochemical changes in the normal metabolism of the body.

EDTA is a chelating agent deliberately used in various fields (paper and pulp industry, detergents industry, food industry, medicine, biomedical laboratories) in order to sequester metal ions that have harmful effects in many industrial processes, as well as in obtaining many products. There are also claims that EDTA appears to be a promising tool to control cadmium pollution in aquaculture.

**Activity 1. Testing the effectiveness of EDTA on cadmium mobilization from tissues of Prussian carps specimens exposed to chronic cadmium acetate poisoning**

Based on EDTA's property to form metal-EDTA complexes, we instituted a study in which Prussian carp specimens with body weight between 35-40 g were exposed for 30 days to sublethal cadmium acetate poisoning (at a dose of 5 ppm in aquarium water), with and without EDTA addition in water (0.05 g/l; 0.1 g/l; 0.15 g/l), to investigate the effect of EDTA on tissue bioaccumulation of Cd.

Spectrometry analyses performed at the end of the experimental period revealed significant increases in Cd concentration in the gills, kidneys, intestine, liver and heart of goldfish, and smaller ones were found in gonads, muscles, skin and brain. As a partial conclusion, the metal studied was more concentrated in the inedible organs of the fish, than at the level of the edible ones - muscle or skin.

The addition of EDTA to the polluted medium at doses of 0.05 g/l, 0.15 g/l and 0.15 g/l gradually led to a reduction in dose-dependent Cd bioaccumulation. Thus, the Cd concentration was below the maximum allowable muscle level (0.02 mg/kg wet weight) when EDTA was introduced into the treatment regimen at a dose of 0.15 g/l.

All these observations highlight EDTA's ability to chelate Cd ions, producing a stable complex that, on the one hand, reduces the absorption of Cd by tissues, and on the other hand, promotes the mobilization of Cd from the body of fish.

**Activity 2. Test EDTA's ability to decrease known cadmium antagonism to some essential macro- and microminerals**

Tissue samples collected from specimens of each group at the end of the experimental period (gill, liver, intestinal, renal, muscle, heart, brain, skin, gonads) were subsequently processed to determine the content of Fe, Zn, Cu, Ca and Mg. The presence of cadmium in water resulted in severe depletion ( $p < 0,001$ ) of Fe, Zn, Cu, Ca and Mg in all tissues analyzed. Cd can disrupt their absorption from water or feed, interfering with binding to transport proteins (e.g. ferritin, transferrin, cysteine, ceruloplasmin), respectively with distribution in fish and implicitly, with homeostatic processes requiring Fe, Zn, Cu, Ca and Mg and finally, intensifying their renal excretion.

In our study, EDTA used as a chelating agent bound Cd while diminishing the antagonistic effect of Cd for essential minerals: Fe, Zn, Cu, Ca, and Mg. The magnitude of EDTA action was dose

dependent, which required further studies of possible risks related to EDTA mobilization, including animal vitals.

**Activity 3. Estimation of potential risks of exposure of Prussian carp specimens to EDTA, on tissue mobilization of mineral elements that have essential roles in performing various cellular functions**

The magnitude of EDTA's use in different fields (industrial, agricultural, medical, domestic), but also its considerable versatility (powerful metal complexing agent and a very stable molecule), have led EDTA to become one of the persistent organic pollutants found in surface waters, as large quantities are released into the environment via wastewater. EDTA itself, or its metabolites, can cause adverse effects targeting the aquatic compartment. At the same time, in fish, macroelements (calcium, phosphorus, magnesium, sodium, potassium, chlorine) and microelements (iron, copper, zinc, manganese) are mineral elements recognized as essential, involved in the formation of skeletal structure, regulation of acid-base balance or osmoreglare.

Continuing the series of investigations, this time regarding the potential risks of using EDTA in aquaculture, we instituted a third experiment in which we added the same concentrations - 0.05 g/l, 0.1 g/l and 0.15 g/l EDTA respectively to the water in which the goldfish were accommodated, for a period of 3 weeks; at the end of the experiment, the concentration of Fe, Cu, Zn, Mn and macroelements Ca, Mg was determined from biological samples collected from animals of control and experimental groups, using atomic absorption spectrometry. The analysis of the variance of the results obtained revealed the existence of significant differences both between tissues and in the treatment regimen adopted.

In control animals, the mineral elements considered in this study showed tissue-specific distribution. In the three experimental groups, on the other hand, their tissue level gradually decreases as the concentration of EDTA in water increases, significant differences being recorded 0.15 g EDTA/l. Added to water to reduce heavy metal toxicity or for other purposes targeting living organisms, it may lead to tissue mobilization of di- and trivalent ions, disrupting mineral homeostasis. We believe that in order to correct deficiencies arising when chelation treatments are necessary, it is necessary to institute mobilized ion replacement therapy.

**Activity 4. Evidence of possible histopathological and morphometric changes induced by chronic exposure of goldfish specimens to increased doses of EDTA**

To indicate the biological effects of xenobiotics on aquatic organisms, both in the natural environment and under experimental conditions, a wide range of biomarkers are used. In addition to measurements/evaluation of physical and chemical parameters, evidence resulting from histocytopathological examinations is recognised as a rapid and particularly valuable tool in assessing the impact of pollutants on fish.

Since reports prior to our study on tissue damage caused by EDTA covered mammalian species and extremely few fish, our research aimed to highlight whether EDTA disrupts specific cellular activity in the species *Carassius gibelio* Bloch, an objective that required histopathological and histomorphometric study.

Compared to the relatively normal appearance of the gills of the individuals in the control group, the lesions reported in the individuals of the experimental groups consisted of cellular epithelial hyperplasia accompanied by vacuolizations (0.05 g/l EDTA), fused gill lamellae and leukocyte infiltrates (0.1 g/l EDTA); severe epithelial degeneration in the experimental group that received EDTA in water at a concentration of 0.15 g/l.

The major characteristic of intestinal histological changes observed in individuals of experimental groups is the slightly damaged appearance of villous epithelium (0.05 g/l EDTA), up to mucosal denudation at villus tip and presence of leukocyte infiltrate (at 0.1 g/l EDTA and 0.15 g/l EDTA).

Hepatic histopathological changes found in individuals in EDTA exposed groups consisted of hepatocyte vacuolization, sign of impaired lipid metabolism (at 0.05 g/l EDTA), isolated necrotic processes at 0.1 g/l EDTA, lipid infiltrations and vascular hypertrophies at 0.15 g/l EDTA. Although liver histopathological changes are considered primary markers of water pollution, they are generally not specific to such situations. Therefore, not all liver damage identified in fish can be used as biomarkers since they appear to be species-specific.

In the case of the kidney, the main common lesions identified on preparations obtained from individuals exposed to increasing doses of EDTA are more or less extensive necrotic processes and vascular congestion regardless of the dose of EDTA used, respectively nuclear pycnotic processes in EDTA2 (0.1 g/l EDTA) and EDTA3 (15 g/l EDTA) groups, respectively.

In the ovary, the presence of atrotic cortical oocytes with strongly thickened chorion was frequently reported in females belonging to the LE3 group.

Damage to gill, liver, intestine, and ovarian tissue, caused by increased doses of EDTA in water, caused a chain of destructive events, with repercussions on respiratory, digestive and reproductive function, as morphometric measurements have also revealed.

#### **Cap. IV. Multidisciplinary approach to detoxifying capacity of natural plant sources and algae with chelating and antioxidant properties in experimental contamination with cadmium and lead in freshwater fish species**

Chelation therapy is the most commonly used therapeutic strategy in case of heavy metal poisoning, which consists of treatment with chelating agents. Synthetic chelating agents such as DMPS, DMSA, EDTA bind heavy metals making them inert, promoting their excretion from the animal body. Despite their rapid action, they can put a huge strain on the body's detoxification systems. Even EDTA, once considered "relatively safe" and much less toxic than DMPS and DMSA, intensifies, as we have shown, the excretion of some bioelements.

However, there are also natural chelating agents that have been proven safe, non-invasive and work as effectively as synthetic chelators, bioactive compounds from natural sources can act as oxygen free radical scavengers or metal chelators.

##### **Activity 1. Highlighting chelating and antioxidant properties of chlorella, coriander and garlic in experimental cadmium poisoning in *Carassius gibelio* Bloch species by comparative evaluation of histopathological aspects of gill, intestinal, hepatic and renal tissue**

The use of natural sources (plant and/or algal) high in phytochemicals with health-promoting, disease-preventing, and treating properties has gained increasing interest around the world, especially due to their availability, low cost, and extremely low number of side effects.

Phytotherapy consists of the integral use of plants, constituent parts or extracts derived from them, as they contain antioxidant compounds and chelates that work synergistically or in combination with each other, resulting in the reduction of oxidative stress. As such, the use of such natural sources with antioxidant and chelating properties is an area of focus now and in the near future.

Histopathological alterations can be used as indicators of the effects of various anthropogenic pollutants on aquatic biota and are a reflection of the overall health of the entire ecosystem population.



Investigating histological changes in fish organs is an accurate way to evaluate the effects of xenobiotic compounds in experimental studies. The initial effects of heavy metal contamination are evident at tissue and cellular level before significant changes in the behaviour or external appearance of fish can be identified.

Starting from these considerations, we proposed the comparative evaluation of the chelating properties of coriander, garlic and chlorella lyophilisate, in cadmium-induced poisoning in Prussian carp specimens, by comparative analysis of histopathological images of gill, intestinal, hepatic and renal tissues.

According to the treatment scheme used in our study, the specimens subjected to poisoning with cadmium chloride at a dose of 10 ppm in water, were additionally administered in the feed - garlic freeze-dried, coriander and chlorella, respectively, in a proportion of 2%, for 21 days.

Tissue alterations identified by microscopic examination of histological pieces were classified according to the degree and extent of lesions (according to histopathological score), as follows: (-) absence of lesions, (+) low frequency, (++) moderate frequency, (+++) severe lesions.

Histological alterations induced by Cd (lamellar thickening and fusion, hypertrophies and vascular congestion) denote the impairment of the respiratory gas exchange barrier with the onset of hypoxia. As a result, the decrease in the rate of oxygen consumption may be associated with malfunctions of osmoregulatory, acid-base or hemodynamic mechanisms.

Lyophilisate garlic, cilantro and chlorella have shown antioxidant and chelating potential against Cd on gill tissue. In individuals of the group who additionally received garlic lyophilisate in the feed, only slight epithelial detachments and cellular edema were observed. The appearance of edema in individuals of this group could serve as a defense mechanism, since separating the epithelium from the lamella increases the diffusion distance that pollutants must travel to pass from the water into the blood. Gill lesions occur less severe and less frequent in the group that received coriander in the feed, in which the lamellar epithelium presents only on small surfaces, detachments or hyperplastic processes. The protective effect of chlorella lyophilisate on the structural and functional gill integrity of fish specimens exposed to sublethal cadmium poisoning was evident, but to a lesser extent compared to garlic and coriander.

Cd contamination affects the liver, inducing pathological lesions, which can be summarized as: cytoplasmic vacuolizations, vascular congestion, lymphocyte infiltrates, fibrosis and necrosis. The incorporation of freeze-dried coriander, garlic and chlorella in feed administered to cadmium-poisoned fish led to some recovery of liver architecture, with mild to medium residual degeneration; Of the three freeze-dried products, coriander powder was found to have the best chelating and antioxidant potential (hepatocytes with few lipid droplets and a small focal necrotic area), while the response to chlorella treatment was less effective, both in terms of severity of lesions observed on preparations obtained from individuals intoxicated with cadmium and receiving added chlorella to the feed, as well as in terms of the frequency of their manifestation.

The histological alterations detected at the level of the intestinal epithelium in individuals of all intoxicated groups suggest that Cd diminishes the efficiency of this barrier, potentially compromising the integrity of the microbiota and implicitly, increasing the risk of local inflammatory processes, infections, allergies and metabolic disorders.

The severe histological changes observed in the kidney tissue of the goldfish specimens after chronic exposure to cadmium chloride were the consequence of the direct action of the toxicant, but also of the side effects caused by the stress response; chronic cadmium poisoning was characterized in the kidney by hypertrophies of the renal tubules, detriment of the nucleus (piknosis), degenerations of the cytoplasm (vacuolization), capillary congestion;

The active principles in garlic and coriander seem to manifest antioxidant and chelating potential favoring the restoration of renal architecture, while chlorella freeze-dried proved less effective than group garlic, without leading to significant differences compared to the coriander.

**Activity 2. Demonstration of morphometric changes induced by chronic exposure of Prussian carps to sub-lethal doses of CdCl<sub>2</sub>**

Histomorphometric analysis of the length of gill blades, gill lamellae and height of intestinal villi of fish subjected to experimental poisoning with CdCl<sub>2</sub> revealed the disruptive action of Cd on respiratory and digestive function. Also the chelating capacity of the active substances in the tested products, as well as their stimulating action on the regenerative potential of the functional elements actively involved in exchanges gaseous and nutritious.

The chelating effect of freeze-dried garlic, coriander and chlorella was manifested as follows: chlorella proved the greatest effectiveness on the length of the gill blades, followed in order by coriander and garlic; addition of coriander - on the length of the gill lamella; chlorella powder - on the height of the intestinal villi, followed by a slight difference between them, garlic and coriander.

**Activity 3. Highlighting the chelating properties of garlic, coriander, chlorella and spirulina in experimental lead poisoning in *Carassius gibelio* Bloch species by comparative evaluation of histopathological aspects of gill, intestinal, hepatic and renal tissue**

Pb induces serious histomorphological changes in vital tissues and, implicitly, disturbances of respiratory, digestive and renal function: massive epithelial and connective degeneration, with shortening to destruction of gill blades and vascular congestion (gills); profoundly altered visceral epithelium (intestine); hepatocellular necrosis, pericentrolubular (liver) necrosis; lesions in glomerular capillaries, enlarged capsular space; hypertrophic renal corpuscles, peritubular edema nephrocytic and interstitial necrosis, (kidney).

The chelating capacity of the active principles of garlic lyophilisate, coriander, spirulina, in the experimental conditions established in this study (dose of lead nitrate administered, period of intoxication, amount of lyophilisate added to feed and respectively, administration period) was manifested differently at the level of tissues analyzed as follows:

- garlic lyophilisate showed good hepatic Pb chelating capacity, was shown to be a good renal Pb binder and did not reduce Pb histopathological changes in the gills and intestine;
- coriander has been shown to be an excellent chelator for Pb in the liver tissue of fish experimentally poisoned with lead nitrate; is able to attenuate the toxic effects of Pb in the kidneys, intestine and gills; as such, it can be administered alongside classical chemical compounds as a potential Pb chelating agent;
- in the group that received chlorella in the feed, the respiratory barrier was only slightly affected by the investigated toxic; the intensity of the destructive action of Pb on the intestinal epithelium could not be counteracted by active chlorella compounds; in the liver, chlorella did not express a chelating effect for Pb; chlorella lyophilisate reduced the toxic effects of Pb in the kidneys; Its lack of chelating action could be due to its rigid and extremely tear-resistant cell wall, plus its associated material – sporopollenin, which could restrict the access of digestive enzymes allowing the proper release and assimilation of intracellular components and, consequently, exerting their chelating and antioxidant effects.
- the active principles in spirulina powder exerted an obvious chelating action for Pb; Consequently, including spirulina in fish feed appears to be a beneficial approach aimed at reducing oxidative stress induced by exposure to toxic agents;

**Activity 4. Evidence of lead antagonism to some essential bioelements in the species *Carassius gibelio* Bloch**

Assessments of the environmental risk of lead in European surface waters concluded that lead is generally not harmful to aquatic organisms at environmental levels, but accidental leakage of waste from various industrial processes or intentional discharges of lead-containing pollutants – in concentrations that can alter water characteristics – affect ecosystem health. Aquatic organisms such as fish accumulate pollutants either directly from contaminated water or indirectly via the food chain. That's why the Agency for Toxic Substances and Disease Registry (ATSDR) has declared lead No. 2 in the top 20 hazardous substances.

An advantage in the fight for survival of fish is the ability to recognize and avoid heavy metals, large amounts of lead in the aquatic environment can cause acute or chronic toxicity, as a result of exceeding the detoxification capacity of the body and its tissue accumulation.

Our investigations were carried out on a number of 180 specimens of Prussian carps exposed for 21 days to sublethal poisoning with lead nitrate, administered at a concentration of 75 ppm in aquarium water, with and without addition to the feed of garlic, coriander, chlorella and spirulina lyophilisate (in proportion of 2% each), and for concentration measurement, tissue synthesis of Cr, Zn, Cu, Mn, Fe, from biological samples (muscles, liver, gills, skin, heart, gonads, brain, intestine) atomic absorption spectrometry was used.

In our study, lead exhibited a strong antagonistic effect to the essential elements Cr, Cu, Zn, Mn, Fe, most likely preventing their uptake from the absorption mucosa (competing with their binding to transport proteins) and/or interfering with their metabolic functions.

Regarding the tissue level of Cr, the antagonistic effect of Pb is so intense that, even if the addition of lyophilisate of garlic and coriander causes to some extent its complexation, Cr remains at relatively low values. The exception is the skin and intestine, where the active principles of garlic lyophilisate and coriander lyophilisate manage to completely counteract the antagonistic effect of Pb to Cr. In contrast, chlorella freeze-dried and spirulina lyophilisate bring tissue Cr levels very close to that of the control group.

Garlic lyophilisate, chlorella, coriander and spirulina additionally administered in the feed, through their active principles, immobilize Pb, thus reducing its antagonism manifested towards Zn. As a result, the tissue level of Zn either remains relatively low compared to controlled individuals in the tissues referred to, or on the contrary, may lead to a tissue distribution exceeding the values recorded in the control group, (in the case of the liver, for example).

In tissues taken from animals that received the four freeze-dried products in feed, the decrease in Cu tissue level occurred less intensely, maximum efficiency in counteracting Pb antagonism against Cu presenting chlorella and spirulina in the heart, liver, kidney and brain; garlic in the brain and testicle, and coriander in the kidney.

The introduction of garlic, coriander, chlorella and spirulina lyophilisate in the treatment regimen contributed to the gradual reduction of the suppressive effect of Pb against tissue transport/uptake/tissue distribution of Mn, maximum efficiency manifesting the four products for the Mn content of the intestine. In the liver, on the other hand, garlic freeze-dried, followed very closely by chlorella lyophilisate, raises Mn levels in control individuals. So are spirulina freeze-dried and cilantro freeze-dried. In the case of gills, spirulina lyophilisate proved to have the most effective metalloprotective effect.

The administration of garlic, coriander, chlorella and spirulina lyophilisate in the diet of fish diminishes the antagonism manifested by Pb vis-à-vis Fe as follows: in the individuals of the group who received additional chlorella in the feed, the values recorded are close to those of the control specimens for liver, heart, intestine and kidney; in individuals of the group who additionally received

spirulina in the feed, the values recorded approach and even exceed those of individuals in the control group: (liver, heart, intestine and kidneys); Garlic freeze-dried not only manages to counteract the mobilization of Fe at the level of liver tissue, but we consider that it also intensifies its capture from the environment and feed; instead, garlic and coriander freeze-dried drugs fail to counteract Fe mobilization in heart tissue, and spirulina - in the testicle and muscle.

The active principles of freeze-dried products used in this study acted to counteract the antagonistic effect manifested by lead on minerals with an essential role in cellular metabolic functions, by preventing their tissue depletion and/or stimulating their uptake at the level of epithelia involved in absorption, which entitles us to affirm that these natural sources can be used as natural agents for detoxification or prevention of heavy metals.

### Cap. V. Synthesis of scientific, professional and academic achievements

The scientific, professional and academic achievements related to the postdoctoral period (1999-2023) can be summarized as follows: publication of a total number of 209 articles (solo trader, first author or collaborator), of which, 20 articles in Thomson Reuters ISI rated journals and ISI proceedings indexed volumes (of which 17 articles in impact factor journals; to 8 articles, having the quality of first author/corresponding author); 114 scientific papers indexed BDI and 75 articles published in the volumes of national and international conferences.

Bibliometric indicators:

*Web of Science profile*: 23 WoS articles; citations: 28; h = 5; <https://o10qgt9n-y-https-www-webofscience-com.z.e-nformation.ro/wos/author/record/AAK-8163-2021>

*Scopus profile*: 19 articles; citations: 57; h = 6;

<https://www.scopus.com/authid/detail.uri?authorId=36659892000>

*Google Scholar profile*: 108 articles indexed in international databases; 500 citations; h-index: 11; <https://scholar.google.ro/citations?user=I2FAsGUAAAJ&hl=en>

I participated in the realization of 18 national research grants/projects won through competition, including research/consultancy projects (minimum value of 10000 Euro equivalent) of which 2 as director and 2 as scientific responsible person.

I have also published in national publishing houses, 4 specialized books, of which 3 as sole author, 4 manuals as single author and a practical works guide as single author.

I am a member of 9 national professional associations and 7 international professional associations.

Regarding the ability to guide students and young researchers, I coordinated more than 50 students for diploma/dissertation papers. In addition to this, I have also guided and helped PhD students in preparing doctoral papers, forming teams within which they managed to publish several scientific papers in journals included in international databases. Last but not least, I coordinated as director 3 educational projects (1 Rose, 2 FDI).

In order to improve my teaching and research activity, I attended several stages of training/specialization, as follows:

- ✚ Specialization course "*Adaptation digestive et metabolique chez ruminants*" organized by U.S.A.M.V.B. Timișoara in collaboration with ENSA Rennes – France, within the francophone education module, 1997

- ✚ Modular course „Current Advances in Cell Biology: Molecular biology techniques: identification of receptor mRNA in primary cells using RNA isolation, transcription and PCR amplification”
- ✚ Tissue culture techniques: culturing human epithelial cells” organizat la USAMVB Timișoara de către „The Childreans Medical and Research Fondation”, Crumlin, Irlanda, 2007
- ✚ Specialization course "Laboratory markers in diagnosis and monitoring of cardiovascular disease therapy", UMF "Victor Babeș" Timisoara, 2010
- ✚ Specialization course "Oxidative stress in cardiovascular pathology", UMF "Victor Babeș" Timisoara, 2011
- ✚ Specialization Program „Virtual interuniversity community for science, technology, innovation and evaluating the intelectual values”. Proiect POSDRU/86/1.2/S/56872, funded by the Social European Funds, Operational sectorial program for human resources development, Universitatea of Vest, Timisoara
- ✚ Training and awareness program in quality assurance in distance learning – ID organized and carried out by Spiru Haret University, in partnership with the Commercial Academy of Satu Mare and TUV Austria-Romania within the Project POSDRU/86/1.2/S607720 funded by the European Social Fund – FS through the Operational Program for Human Resources Development 2007-2013
- ✚ Career Guidance Counselor specialization course, 2016
- ✚ Trainer specialization course, 2017
- ✚ Professional Skills Evaluator specialization course, 2018
- ✚ Specialization Course Supplier Evaluator and Training Programs, 2018

The scientific research, synthetically presented and documented in this habilitation thesis, is completed with a series of other topics carried out in collaboration with teachers and researchers from the Faculty of Bioengineering of Animal Resources and from the "King Mihai I" University of Life Sciences in Timisoara, as well as from the "Victor Babeș" University of Medicine and Pharmacy in Timișoara.

Based on the grants won through the competition and those I worked for as a collaborator, I contributed to equipping the laboratory of the discipline of Physiology and the Learning Center of the Faculty of Biongenieria of Animal Resources, with simulation programs of Physiology, specific reagents, computer technology, specialized books. All these had as main purpose the diversification of means leading to increasing the level of professional training of students, always focusing on student-centered education.

### **CAP. VI. Plans for the evolution and development of my own professional, scientific and academic career**

The career development perspective is based on two main objectives, namely: deepening and developing the teaching and research activity, but also expanding the areas of expertise, with the development of research activity.

My research will continue to focus on aspects related to the physiology of farm animals, including those aimed at directing the great functions of the organism of farm animals. The lactogenic function of the mammary gland, respectively the growth and differentiation of tissues; highlighting metabolic, biochemical, hematological and tissue histopathological changes induced by chronic heavy metal poisoning in freshwater fish species. Testing of vegetal active principles with chelating and

antioxidant properties in chronic heavy metal poisoning. The above-mentioned are the fields on which my entire professional activity was based and in which I obtained the title of "doctor of science – Ph.D". As well as all academic didactic degree up to that of associate professor.

Through my entire professional, scientific and academic activity, I want to contribute to increasing the scientific reputation, competitiveness and national and international visibility of the Faculty of Bioengineering of Animal Resources and, implicitly, of the University of Life Sciences "King Mihai I" from Timișoara.

**Assoc. Prof. Dr. Eng. Nicula-Neagu Marioara**