Summary of professional accomplishments

Scientific output and achievements

Sylwia Terpiłowska, PhD

Department of Surgical Medicine with the Laboratory of Medical Genetics
Collegium Medicum
Jan Kochanowski University in Kielce

Kielce 2023

1. Name and surname

Sylwia Terpiłowska

2. Diplomas, degrees conferred in specific areas of science, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation

2000 Master of Biology, speciality: microbiology, The Faculty of Biology and Geo-Science, Maria Curie-Sklodowska University, Thesis title: „*Spontaneous and induced production of cytokines by bone marrow stroma in patients with multiple myeloma and acute myeloid leukemia*". Supervisor: prof. dr hab. Martyna Kandefer-Szerszeń

2007 Postgraduate Studies in European Fund Management, The Faculty of Social Sciences, The John Paul II Catholic University of Lublin

2008 Doctor of veterinary sciences, specialization: immunology, The Faculty of Veterinary Medicine, The University of Warmia and Mazury in Olsztyn, Thesis title: „*The influence of chromium and iron on fibroblasts metabolism and cell-mediated immunity in mice*" Supervisor: Andrzej K. Siwicki.

2010 Postgraduate Studies in Internal Audit, The Faculty of Law, Canon Law and Administration, The John Paul II Catholic University of Lublin

2011 Postgraduate Studies in Protection of Intellectual Property, The Faculty of Biology and Biotechnology, The University of Maria Curie Sklodowska, Lublin

2012 Postgraduate Studies in Molecular Diagnostics, The Faculty of Agrobioengineering, The University of Life Sciences, Lublin

3. Information on employment in research institutes or faculties/departments or school of arts

From 2021 until now Assistant Professor Department of Surgical Medicine with the Laboratory of Medical Genetics, Collegium Medicum, Jan Kochanowski University in Kielce

From 2000 until 2021 at The John Paul II Catholic University of Lublin:

2000–2005 Assistant, Department of Cell Biology, Institute of Environmental Protection

2005–2010 administrative officer at the International Relations Office

2010–2021 Assistant Professor, Head of the Laboratory of Environmental Biology, Institute of Environmental Protection at Off-Campus of KUL at Stalowa Wola

March-October 2021- Assistant professor at the Institute of Health

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

Title of the achievement:

Toxicity and mechanisms of action of selected transition elements with particular reference to the interaction of chromium(III) with other elements - *in vitro* studies.

Papers included in the monothematic publication cycle

The series of thematically related scientific articles constituting the scientific achievement consists of 6 papers. In all of them, I am the first author and the corresponding author.

The total impact factor of the single-topic series of papers is 19,202 (Impact Factor according to the date of publication), while the total number of points from the list of journals from the Ministry of Science and Higher Education is 540. For full bibliographical details, see *Annex List of scientific or artistic achievements*.

	Publikacja	**IF**	**Points of MSHE**
4.1	**Terpiłowska S.**,*, Siwicki A. K., 2017, Chromium(III) and iron(III) inhibits replication of DNA and RNA viruses, Biometals, 30(4): 565–574	2.478	30
4.2	**Terpilowska S.**,* Siwicki A.K., 2018, Interactions between chromium(III) and iron(III), molybdenum(III) or nickel(II): cytotoxicity, genotoxicity and mutagenicity studies. Chemosphere, 201, 780-789	5.108	35
4.3	**Terpilowska S.**,* Siwicki A.K., 2019, Cell cycle and transmembrane mitochondrial potential analysis after chromium(III), iron(III), molybdenum(III) or nickel(II) and their mixture treatment. Toxicology Research, 8(2), 188-195	1.593	35
4.4	**Terpilowska S.**,* Siwicki A.K., 2019, Pro- and antioxidant activity of chromium(III), iron(III),	3.723	100

	molybdenum(III) or nickel(II) and their mixtures, Chemico-Biological Interactions, 298, 43-51		
4.5	Terpiłowska S.*, Pięta E., Roman M., Paluszkiewicz C., Kwiatek W.M., Spectroscopic imaging to assess biochemical alterations in liver carcinoma cells exposed to transition metals, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2023, 303, 1232	4.4	140
4.6	**Terpiłowska S.*,** Rafińska K., Gołębiowski A., Kowalkowski T., Buszewski B., 2023, The influence of chromium, iron, nickel, molybdenum and cobalt on the concentration of selected micro- and macroelements and cell morphology, Biological Trace Element Research, 30(4):471-488	1.9	200

***** - corresponding author

Presentation of the scientific aims of the work and the results achieved, discussing their potential applications.

Introduction

Chromium, iron, nickel and molybdenum belong to the group of trace elements. The human and animal organisms need them only in small amounts. The levels of microelements (trace elements) in the organism are below 1 μg/g of wet tissue. Despite the low concentration of microelements in body fluids and tissues, trace elements are involved in many cellular pathways. They get into the bodies of animals and people through the respiratory tract, through the skin and to the greatest extent through the digestive tract. They get together with the diet, but also in the form of vitamin and mineral preparations, dietary supplements or energy drinks, whose supply has recently increased significantly. In recent years, more and more elements have been introduced into human and animal organisms along with large-scale biomaterials. Biomaterials are commonly characterized as materials used to construct artificial organs, rehabilitation devices, or

implants to replace natural body tissues. More specifically, biomaterials are materials that are used in close or direct contact with the body to augment or replace faulty materials (Bauer et al., 2013; Patel et al., 2012). It should be noted that these materials contain many elements whose full metabolism at the cellular level is not fully understood. This is important because elements remaining in the human body may have toxic effects at organ, tissue or cellular level. In addition, the potential for interaction between elements at the cellular level is completely unknown.

Chromium is an element commonly occurring in nature. Chromium exists primarily in two valence states: trivalent (Cr III) and hexavalent (Cr VI). It has been shown that Cr(VI) is cytotoxic, genotoxic and carcinogenic, while Cr(III) appears to be relatively non-toxic (Rudolf and Červinka, 2003). The hexavalent chromium compounds are more than 1000-fold more toxic than the trivalent compounds (Hininger et al., 2007). Chromium(VI) compounds enter cells via sulphate ion transporter (Figgitt et al., 2010). When Cr(VI) enters the cells it is reduced to Cr^{5+}, Cr^{4+} and finally to Cr^{3+} (Valko et al., 2006). Additionally, the reduction of chromium(VI) depends upon the pH and occurs faster in acidic pH than in basic pH (Langard i Costa, 2015). These intermediates can react with DNA. Moreover, during these processes, Reactive Oxygen Species (ROS) are produced and interact with various cell organelles. Cr^{3+} penetrates the cell membrane by passive transport, endocytosis or phagocytosis (Eastmond et al., 2008). In addition, Cr(III) can be transported into cells with transferrin and activate the insulin receptor. Approximately 80% of the chromium in the blood is bound to transferrin. Under physiological conditions, this protein binds ions, allowing them to be transported into cells. Transferrin preferentially binds iron ions (Fe^{3+}). Transferrin is transported into cells by endocytosis. It has been shown that Cr^{3+} can also bind to transferrin replacing Fe^{3+} ions. In response to an increase in plasma insulin concentration, the transferrin receptor (Tf-R) in insulin-sensitive cells migrates from vesicles to the plasma membrane. Transferrin, which contains two bound metal ions (one Cr^{3+} ion and one other metal cation (M)), binds to the receptor and is internalized by endocytosis. The pH of the resulting vesicle

is lowered by ATP proton pumps, resulting in the release of Cr^{3+} from transferrin. The chromium(III) released from many transferrin molecules is sequestered by apochromodulin, resulting in its conversion to chromodulin. This in turn is required for insulin receptor activation (Vincent and Edwards, 2019). Inside the cells, chromium(III) may interact with microfilaments, mitochondria, lysosomes and nuclei. Cr(III) compounds can bind directly to DNA *in vitro*, forming Cr-DNA adducts and DNA-DNA crosslinks (O'Brien et al., 2003). What is more, Cr(III) has been shown to be able to increase the catalytic activity and decrease the fidelity of DNA polymerase (Galaris and Evangelou, 2002). Chromium(III) is essential for proper insulin functioning (insulin-receptor activation) and is required for normal protein, fat and carbohydrate metabolism (Baghi et al., 2002).

Iron is an essential element that plays a vital role in many cellular processes (Srai et al., 2002). It is the key component of many cellular enzymes such as oxidases, catalases, peroxidases, cytochromes, nitric oxide synthases, ribonucleotide reductases and aconitases. These enzymes are involved in many cellular processes, i.e. DNA and RNA synthesis, oxygen and electron transport and cell proliferation (Lieu et al., 2001). Its activity in biological systems is related to its ability to change its oxidation state. Particularly important is its involvement, in the Haber-Weiss reaction.

$Fe^{3+} + O_2^{-} \rightarrow Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} + Fe^{3+}$

$O_2^{-} + H_2O_2 \rightarrow OH^{-} + OH^{\cdot} + O_2$

On the other hand, Fe^{2+} and Fe^{3+} ions can be toxic, especially because of its property to form insoluble salts and to catalyze formation of free radicals (Hirsh et al., 2002a; Hirsh et al., 2002b). Iron mediated formation of ROS leading to DNA and lipid damage, cause cell death or cancer (Valko et al., 2006). The products of DNA damage are strand breaks, oxidatively modified bases and DNA-protein crosslinks. The damages include a decrease in membrane polyunsaturated fatty acids content, inhibition of thiol-dependent

enzyme activities, lowered ATP and enhanced lysosomal fragility. Moreover, inhibition of the activity of NADH-cytochrome c oxidoreductase (complex I-III) and succinate dehydrogenase was observed (Hirsh et al.., 2002a; Hirsh et al., 2002b)

Nickel exists in five valences or oxidation states of -1, +1, +2, +3, and +4, +2 being the most common. Nickel compounds are taken up by mammalian cells, where nickel ions are released and then they can induce cytotoxicity, apoptosis, chromosomal aberrations and morphological transformation. Moreover, nickel has been found to induce chromosome deletions and genomic instability. Nickel is also mutagenic. It has been found that nickel induces epigenetic gene silencing and histone modifications. Nickel can increase H3K4me3 and decrease H3K9me3 histones (Klein i M. Costa, 2015; Arita et al., 2012).

Molybdenum is an element necessary for the normal functioning of cell metabolism. The most important oxidation states for molybdenum are +2, +3, +4 and +6 (Tallkvist and Oskarsson, 2015). Molybdenum plays a particular role in the active sites of metalloenzymes: xanthine oxidoreductase, aldehyde oxidase, mitochondrial amidoxime reductase component and nitrate reductase xanthine oxidase, xanthine dehydrogenase and sulfite oxidase (Mendel, 2013).

Objectives of the work

In view of the increasing supply of the elements, their involvement in cell metabolism and their ability to induce cell death (apoptosis), which can lead to the induction of many diseases including cancer, the present study attempted to determine their metabolism. The aim of the study was to determine the effects of chromium, iron, nickel and molybdenum on the metabolism of normal and cancer cells and to determine the interactions between chromium and iron, chromium and nickel and chromium and molybdenum. The overall objectives were met through specific objectives:

a) determination of the IC_{50} doses of chromium chloride, iron chloride, nickel chloride and molybdenum trioxide using cytotoxicity assays.

b) determination of the genotoxicity and its mechanisms of chromium chloride, ferric chloride, nickel chloride and molybdenum trioxide using comet and micronucleus tests.
c) determination of the mutagenicity mechanisms of chromium chloride, ferric chloride, nickel chloride and molybdenum trioxide using the AMES assay.
d) determination of the mechanisms of apoptosis induction by chromium chloride, ferric chloride, nickel chloride and molybdenum trioxide using assays to determine the formation of oxygen free radicals and their products in the cell and the activity of the antioxidant system.
e) determination other mechanisms of apoptosis induced by chromium chloride, iron chloride, nickel chloride and molybdenum trioxide using spectroscopic techniques: Raman and FTIR and ICP-MS.
f) 6 determination of the antiviral activity of chromium chloride and ferric chloride.
g) identify the type of interaction between chromium chloride and ferric chloride or nickel chloride or molybdenum trioxide

Materials and methods

I. Cyto- and genotoxicity, mutagenicity, oxidative stress, cell cycle phase analysis and mitochondrial membrane potential studies

The studies were conducted on two cell lines: Mouse embryo fibroblasts BALB/3T3 clone A31 cells (American Type Culture Collection CCL-163) and Liver cancer HepG2 cells (American Type Culture Collection HB-8065). The cells were cultured in media supplemented with chromium chloride or iron chloride or nickel chloride or molybdenum trioxide within a range of concentrations from 100 to 1400 μM. In order to perform interaction studies the cells were exposed to the mixture of microelements 200 μM of chromium chloride plus 1000 μM of iron chloride or nickel chloride or molybdenum trioxide and, in the other case, supplemented with 1000 μM of chromium chloride plus 200 μM of iron chloride or nickel chloride or molybdenum trioxide. After 24 hours of incubation, the following assays were performed:

a) cytotoxicity: the MTT reduction assay, the LDH release, the Neutral Red Uptake assay (NRU),
b) genotoxicity: Comet and Micronucleus assays
c) The cell cycle phase determination analysis
d) Mitochondrial transmembrane potential (MTP) analysis
e) DCFDA Cellular ROS detection assay, TBARS assay
f) Antioxidant enzymes activity: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidise (GPx) assays.

Moreover, AMES assay was performed for microelements used alone and in combinations, with the use of *Salmonella typhimurium* TA98 andTA100.

II. Investigations of the mechanisms of apoptosis using Raman and Fourier-transform infrared spectroscopy (FTIR) and ELISA.

Studies were conducted on the liver cancer cell line HepG2 cells (American Type Culture Collection HB-8065). Cells were incubated with chromium chloride, iron chloride, nickel chloride and molybdenum trioxide in the concentration range 100-2000 μM. After 24 hours of incubation, the concentration of the following caspases was determined: 3, 6, 8, 9 and 12.

In addition, cells were cultured on CaF_2 slides. The cells were incubated with chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride at concentrations of 1000 and 2000 μM each. After 24 hours of incubation, the cells were fixed and analysed using Raman and FT-IR spectroscopy.

III. Testing of chromium, iron, nickel, molybdenum, calcium, magnesium, sodium and potassium levels using ICP-MS. Observations of cell morphology using a scanning electron microscope (SEM).

Studies were conducted on three cell lines: L929 (mouse fibroblasts), Caco-2 (epithelial cells) and HepG2 (liver cancer cells). Cells were incubated with chromium chloride, ferric chloride, nickel chloride and molybdenum trioxide at concentrations of 200 and 1000 μM. To determine the type of interactions, cells were incubated with mixtures of the elements: chromium chloride at a concentration of 200 μM with iron chloride, nickel chloride or molybdenum trioxide at a concentration of 1000 μM. In the second case, a mixture of

chromium chloride at a concentration of 1000 μM with iron chloride, nickel chloride or molybdenum trioxide at a concentration of 200 μM was used. After 24 hours of incubation, the cells were mineralised and subjected to ICP-MS analysis. On the other hand, the cell observed under the SEM microscope was fixed and analysed under the microscope.

IV. Antiviral activity studies

Tests were carried out on two cell lines: HEp-2, on which Herpes Simplex Virus HSV-1 was propagated, and line BT (turbinate cells), on which Bovine Viral Diarrhoea Virus (BVDV) was propagated. In the first step, the cytotoxicity of the chromium chloride and iron chloride concentrations used was determined in the concentration ranges 100 - 1200 μM on both cell lines, using the MTT reduction assay. In the next step, cells of both lines were incubated for 24 hours with chromium chloride or iron chloride at concentrations of 100, 200, 400, 600, 800, 1000 and 1200 μM or simultaneously in the following mixtures: (1) chromium chloride at 200 μM plus iron chloride at 1000 μM, (2) chromium chloride at 1000 μM plus iron chloride at 200 μM,(3) chromium chloride at 400 μM plus iron chloride at 800 □M and (4) chromium chloride at 800 μM plus iron chloride at 400 μM. HSV-1 or BVDV virus was added to each sample. For HSV-1 virus, the culture was conducted for 2 days, while for BVDV the culture was conducted for 5 days. After this time, DNA or RNA was isolated, respectively. Virus identification was then carried out by Real-Time PCR for HSV or qRT PCR for BVDV.

Results and discussion

The first problem in *in vitro* studies is the choice of the appropriate dose used for the studies. In the case of *in vivo* studies, the Recommended Daily Allowance (RDA) or Adequate Intake (AI) has not been specified for many microelements. For example, the available literature gives several values determining the body's need for chromium(III):

- ESADDI (Estimated Safe and Adequate Daily Dietary Intakes for Chromium) is an essential daily dose of chromium, which for different age groups ranges from 10 to 200 μg depending on age.

- RDA (Recommended Daily Allowance) - recommended daily requirement, which is, as stated by various authors, 30-100 g or 50-200 g/day.
- Optimal Daily Allowance (ODA) – the optimal daily requirement, which is 0.200 mg.
- Reference Dose (RfD) - the reference dose, which is 1-1,5 mg/kg m.c./day.
- Adequate Intake (AI) - sufficient intake, which depends on many varies from 2.0-11 µg/day.
- Reference Dietary Intake (RDI): 120 g/day (Berner, 2004, Vincent, 2001).

On the other hand, for iron are assigned:

- Recommended Dietary Intakes (RDI), which is, depending on age, 7-27 mg (Swanson, 2003).
- Recommended Daily Allowance (RDA) - recommended daily requirement, which is: 19-51 years old: 10 mg men, 15 mg women over 51 years old 10 mg.

However, those doses, differ from one another and were also prescribed in *in vivo* studies. The effective absorption in the intestine is low. For example, for chromium, the absorption is approximately 0.4% for chromic chloride, and for organic compounds: picolinate and nicotine, approx. 5%. Iron absorption is approximately 2-15%. Fe^{2+} salts are better absorbed than Fe^{3+}. The absorption also depends on the compounds of the given element which was used. The absorption also depends on the composition of the diet, the content of lipids, proteins or other interactive substances that may enhance or weaken its absorption. The above doses refer to intestinal absorption, while the elements reach individual cells in different concentrations. The appropriate doses for the *in vitro* studies were selected on the basis of literature data and previous studies. In addition, the correctness of the used doses was confirmed in work 4.6. Using the ICP-MS technique, the levels of chromium, iron, nickel and molybdenum were measured after incubation of cells with the compounds studied. The element absorption level for chromium ranged from 0.0016 to 0.003 per cent, for iron from 0,0002 to 0.01 per cent for nickel from 0.0009 to 0,005 per cent and for molybdenum from 0,008 to 0,001 per cent. Such a low level of absorption of elements is associated with the passive forms of transport into the cells of the elements studied and their

formation in the cell culture medium of complex compounds, which are very poorly transported to the cell. Chromium(III) in the presence of the serum has been shown to interact with albumin, transferrin and antibodies to form complexes that cannot be transported through the cell membrane. (Tkaczyk et al., 2010).

The use of a wide range of tests to study of cell metabolism was used to determine the effect of individual micronutrients and their mixtures on the functions of normal and cancer cells.

Cell lines are an excellent tool for screening toxicity tests of various biologically active substances. They are used in the first stage of assessing the biological activity of various compounds and determining their potential toxicity. They allow limiting the use of laboratory animals for testing in the first stages of research of new substances. According to the principle of 3R (Reduction, Replacement, Refinement), cell lines are used to assess the toxicity of substances, but many of the methods have validation carried out by European Union Reference Laboratory for Alternatives to Animals Testing EURL ECVAM, which replaces animal testing. One of the frequently used cell lines is mouse fibroblasts. They are used in a validated method for the determination of acute toxicity and phototoxicity with the use of neutral red. The BALB/3T3 cell line was chosen for our investigation because it was suggested as a cellular model for studying the morphological and biochemical changes induced by biometals (Mazzotti et al., 2002). Human hepatoblastoma HepG2 cells have been well characterized and extensively used as, an *in vitro* toxicity model (Fang and Beland, 2009; Schoonen et al., 2005). This line is recommended in the OECD protocol to perform the micronucleus test.

All the used assays were performed according to EURL ECVAM, OECD protocols (Guideline no. 471, Guideline no. 487) and Polish Standards (PN-EN ISO 10993-3:2008).

For toxicity evaluations, mitochondrial function (MTT reduction assay), cell membrane integrity (lactate dehydrogenase (LDH) release assay), and lysosome integrity (neutral red uptake (NRU)) assay were used. The use of

these three assays is very useful for examining the three different endpoints in the same cells at the same time (Chiba et al., 1998).

In this study, the genotoxicity in HepG2 and BABL/3T3 cells using two end-points: chromosome breakage or loss demonstrated by the micronucleus test and primary DNA damage (single- and double-strand breaks and alkali-labile sites) demonstrated by the comet assay were investigated. The comet assay is a common technique for measurement of DNA damage in individual cells. Under an electric field, damaged cellular DNA (containing fragments and strand breaks) is separated from intact DNA, yielding a classic "comet tail" shape under the microscope. Extent of DNA damage is visually estimated by comet tail measurement (Pu et al., 2015). The *in vitro* micronucleus test detects chromosome breakage and loss by measuring the formation of micronuclei. These are small membrane-bound fragments or whole chromosomes, which are unable to attach to the spindle at mitosis and appear as small bodies within the cell. The proportion of cells that have undergone cell division and suffered chromosome breakage or loss, resulting in micronucleus formation can then be counted, giving a representation of the genotoxicity of the test item (Fenech, 2008). The principle of the AMES test is based on the hypothesis that the application of mutagen leads to mutations in many genes including the defective gene, and some of those mutations cause the reverse of ability to synthesize histidine (reverse mutations). After the subjection of his- *Salmonella.* Typhimurium to the tested substance, the bacteria are placed on a medium that does not contain histidine. There are several different mutant strains of *S. typhimurium* that have different mutations of their DNA: 1) TA 100- its mutagenic specificity is similar to that of the base-pair substitutions mutagen tester strain, 2) TA 98- its mutagenic specificity is similar to that of the frameshift mutagen tester strain (Kozłowska et al., 2012).

The cell cycle consists of five phases: G0, G1, S, G2 and M. Cell cycle progression depends on the activity of cyclins and cyclin-dependent kinases (cdks). The cyclin-cdk complexes are activated by phosphorylation *via* cyclin-activating kinases (CAKs). These complexes are necessary to pass subsequent

checkpoints in the cell cycle. Such protection is provided by control points G1 and G2 during the cell cycle. Their defeat by the cells whose DNA is free from mutations or other damage lets them enter mitosis (Le and Richardson, 2002).

The analysis of mitochondrial potential serves to assess the pro-apoptotic activity of biologically active substances. During apoptosis, several key events occur in mitochondria, including the release of cytochrome c, changes in electron transport and loss of mitochondrial transmembrane potential.

Many active biological substances induce Reactive Oxygen Species (ROS) production inside the cell which is a physiological phenomenon in the cell. However, when antioxidant enzymes are insufficient to neutralize ROS, the damage of individual cellular structures may take place. ROS is a group containing many different compounds. In the research presented below, ROS generation was determined using 2',7'–dichlorofluorescin diacetate (DCFDA). In the other used assay, a concentration of malondialdehyde was performed. Polyunsaturated fatty acids, containing two or more double bonds are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides. This process is called lipid peroxidation. It is a free radical-mediated chain reaction involving initiation, propagation and termination. The first step - initiation is started by the abstraction of a hydrogen atom from a polyunsaturated fatty acid moiety of membrane phospholipids by the attack of reactive species. The fatty acid radicals formed in the first step react with the lipids and generate new free radicals. The propagation phase can repeat many times until it is stopped by chain-breaking antioxidants. The breakdown of lipid peroxidation products results in the formation of malondialdehyde (MDA). Malondialdehyde level is commonly known as a marker of lipid peroxidation (Grosicka-Maciąg, 2011).

Living organisms have developed complex antioxidant systems to counteract ROS and to reduce their adverse effects. These antioxidant systems include enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidise (GPx). Superoxide dismutase, the enzyme catalyzing the dismutation of superoxide radicals to molecular oxygen and hydrogen

peroxide, is an antioxidant agent. The hydrogen peroxide is later converted to molecular oxygen and water by catalase. Glutathione peroxidase catalyzes the reduction of hydroxyperoxides including hydrogen peroxide and lipid peroxidative products, by reduced glutathione finally protecting cells from their damage. SOD in conjugation with CAT and GPx scavenge both intracellular and extracellular radicals and inhibit lipid peroxidation (Czajka, 2006).

The tests described above are a classic methods to examining the biological functions of cells after exposure to various substances. There are more and more reports on the use of modern research techniques, i.e. Raman spectroscopy and FTIR spectroscopy, to determine the mechanisms of action of various substances. This is a new tool enabling the study of cell metabolism. The available literature contains works on the identification of various types of cancer cells and tissues using spectroscopic methods. Moreover, these methods were used to study the impact of metallic nanostructures on cell metabolism, to determine IC_{50} concentrations, or to determine the interactions of selected elements with cell structures in various diseases. However, there is no data on determining the effect of metals on the identification of apoptosis mechanisms (Rauwel, 2020, Lv, 2022, Berntsson, 2023, Huang, 2023). In this study, spectroscopic methods were used to identify the mechanisms of apoptosis.

The ICP-MS technique was used to identify the concentration of the elements used in the cell. It was also used to determine the concentrations of calcium, magnesium, sodium and potassium inside the cell after the treatment of the tested elements. This technique is becoming more and more widely used in cell biology to determine changes in ion concentration and their impact on cell metabolism.

Another important aspect discussed in the research was the possibility of exposure to microelements inhibiting the replication of DNA and RNA viruses. The HSV-1 virus was selected for the study, which causes many human diseases and is also a good research model for DNA viruses. However, the BVDV virus is the cause of infections of the digestive system of cattle and

infections with this virus reduce the profitability of breeding, but also increase the frequency of abortions and low birth weight of animals. It was a model RNA virus.

Chromium is an element commonly occurring in nature. Its supply has increased in the recent years. Dietary supplements containing chromium(III) have become very popular. In this study cell viability was stimulated in MTT assay after chromium chloride at concentrations of 100 and 200 μM treatment. The present study demonstrates that chromium chloride (at concentration range of 400-1400 μM) induces dose-dependent decrease of cell viability in normal cells (BALB/3T3) and cancer cells (HepG2) assessed by MTT reduction assay, LDH release assay and NRU assay. The results obtained from MTTT and NRU assays in both cell lines show that HepG2 cells are more sensitive when compared to the BALB/3T3 cells. Basing on IC_{50} values, it can be concluded that cell membrane may be the first organelle in which the effect of chromium chloride is observed. The damage of mitochondria and then lysosomes follows cell membrane damage in both cell lines (paper no. 4.2). The above-mentioned observations confirm the study conducted in the flow cytometric study - analysis of mitochondrial transmembrane potential. In this study the decrease of mitochondrial transmembrane potential in both cell lines after chromium chloride at the concentration range of 100-1000 μM treatment was observed (paper no. 4.3). This supports the observation that ROS are responsible for chromium(III)-induced DNA and mitochondria damage.

The negative effect of chromium chloride was observed also in genotoxicity and mutagenicity tests. A statistically significant increase in DNA damage after chromium chloride treatment in both cell lines was observed. In the available literature, it has been demonstrated that Cr(III) compounds can bind directly to DNA *in vitro*, forming Cr-DNA adducts and DNA-DNA crosslinks (O'Brien et al., 2003). Electropositive trivalent chromium ions are bound to negatively charged phosphate groups and to the base guanine. Therefore, DNA-DNA crosslinks are formed due to phosphate-chromium-phosphate and guanine-chromium-guanine links (Snow and Xu, 1991).

Moreover, positive results obtained from comet assay (paper no 4.2) confirm that chromium chloride induces DNA damage (single- and double-strand breaks and alkali-labile sites). Consequently, the DNA structure gets destructed. DNA destruction should be removed by repair enzymes before replication. In our study DNA decreases in the G0/G1, while it increases in the G2/M phase. It can be concluded that trivalent chromium arrests the cell cycle in the G2/M phase. Dai et al. (2009) show that trivalent chromium increases the frequency of mutations: substitutions, transversions, deletions and insertions. Investigations conducted in paper no. 4.2 shows that chromium chloride evokes frameshift mutation more often than base–pair substitution mutation. Moreover, aneuploidy peak (subG1) of DNA content was observed. This peak may represent cells that escaped mitotic arrest, and which replicate as multinuclear cells without dividing. Giant and multinuclear cells observed in microscopic analyses are the effect of this process (paper no. 4.2). What is more, apoptotic cells were observed. The micronucleus assay performed with the use of chromium chloride shows statistically significant induction of chromosomal aberrations in all tested microelements in both cell lines (paper no. 4.2). These results are confirmed by microscopic observations: multinuclear cells were observed.

Observations conducted by other authors suggest that the destruction of many cell organelles, i.e. cell membrane, mitochondria, lysosomes, nucleus, spindle apparatus, disorders of cell metabolism, i.e. respiratory and proliferation processes may be induced by reactive oxygen species. The influence of chromium chloride on ROS and MDA production and activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) is to be investigated in paper no. 4.4. The increase of ROS and MDA after chromium chloride at concentration of 400 μM and above this concentration in both cell lines was observed. Moreover, the increase of SOD and CAT activity after incubation with chromium chloride at concentration of 100 and 200 μM were observed, whereas this activity decreases after incubation with chromium chloride above the concentration of 400 μM in both cell lines. What is more, glutathione peroxidase activity statistically significantly decreases

depending on the dose, after treatment with chromium chloride. These results are in agreement with other investigations. An increase in activity of SOD and CAT after chromium(III) treatment at low concentrations was observed (Długosz et al., 2012; Chen et al., 2018). However, at higher concentrations, decreased activity of this enzyme was observed. The dysfunction of catalase activity may be associated with the destruction of the active site of CAT by the excess of Cr(III) (Chen et al., 2018). In my study (paper no. 4.4) the decrease in antioxidant enzymes activity was correlated with the increase in ROS concentration.

High concentrations of ROS and inhibition of antioxidant enzymes activity induce apoptosis. Cells showing characteristic morphological changes, i.e. chromatin condensation or the formation of apoptotic bodies, were observed in work 4.2. Therefore, in work 4.5 we decided to investigate the mechanism leading to apoptosis. The concentration of kinases activated in the apoptosis was determined. Incubation of cells with chromium chloride resulted in an increase in the concentration of caspases 3, 6, 9 and 12. An increase in the concentration of initiator caspase 9 and executioners caspases: 3 and 6 during activation of the intrinsic pathway involving mitochondria was observed. This confirms the observations contained in papers 4.2, 4.3, and 4.4. An increase in ROS concentration results in the inhibition of lipid B-oxidation in the mitochondrion, leading to the formation of lipid droplets. Additionally, an increase in the concentration of caspase 12 was observed. It is involved in the induction of apoptosis associated with damage to the endoplasmic reticulum (RE). This organelle is a reservoir of lipids. Its damage causes the release of lipid droplets into the cytoplasm. The lipid droplet components observed in this study are oleic acid derivatives (triolein and cholesteryl oleate). They were observed in studies using Raman and FT-IR spectrometry (paper 4.5).

As the research performed in work 4.6 showed, there were other mechanisms leading to the induction of apoptosis in cells. Inhibition of the balance of Na^+ and K^+ concentrations was another mechanism that was related to the induction of apoptosis. These elements are crucial for

maintaining the resting cell membrane potential. Research by Yurinskaya et al. showed that disruption of the activity of the sodium-potassium pump causes a change in the conformation of membrane lipids and the formation of apoptotic bodies. Changes in the cell membrane were observed both in the LDH release test and in fluorescence microscopy preparations (paper 4.2) and in electron microscope preparations (paper 4.6). Also, an imbalance of other elements in the cell leads to apoptosis. The endoplasmic reticulum and mitochondria are involved in maintaining Ca^{2+} homeostasis. Damage to the RE leads to the release of Ca^{2+} ions into the cytoplasm. RE damage and lipid droplet formation were found in paper 4.5. This was confirmed in work 4.6, where an increase in Ca^{2+} concentration in the cytoplasm was observed. This phenomenon also has a negative impact on the formation of karyokinetic spindle elements. Symptoms of these changes may include disruption of the cell cycle and the formation of micronuclei, which were observed in work 4.2. The imbalance of magnesium leads to apoptosis. It can induce apoptosis by the influence on mitochondrial metabolism, induction of cytochrome c release and activation of caspases 3 and 9. Their increased concentration was found in paper 4.5.

The next analyzed microelement was iron. Iron is an essential element playing a vital role in many cellular processes (Srai et al., 2002). Fe is involved in many cellular processes, i.e. DNA and RNA synthesis, oxygen and electron transport and cell proliferation (Lieu et al., 2001). In the study presented in paper no. 4.2 iron chloride induces cytotoxicity. However, HepG2 cells are more sensitive when compared to the BALB/3T3 cells in MTT and NRU assays. In these assays reduction of cell viability was higher in cancer cells than in normal cells after iron chloride treatment. In both mentioned assays, in HepG2 cells, cell viability decreased above the concentration of 100 μM. However, the decrease of cell viability at dose 800 μM and above that in BALB/3T3 cells was observed. It may suggest that the mitochondria of cancer cells are more sensitive when compared to the mitochondria of normal cells after incubation with iron chloride. These observations are confirmed in paper no. 4.3. In the paper mentioned above a concentration-dependent statistically significant

decrease in the level of MTP was observed in both cell lines after exposure to iron chloride at the concentration range of 100-1000 μM. The results obtained from both cell lines show that HepG2 cells are more sensitive when compared to the BALB/3T3 cells. In the LDH release assay, which determines the sensitivity of the cell membrane to the test compound, iron chloride showed similar toxicity in both lines. The results obtained from both cell lines show that both cell lines are similar in their sensitivity to iron chloride. The results obtained from cytotoxicity assays and MTP and investigations performed by other authors show that the toxic effect of Fe(III) is caused by ROS (Eid i wsp., 2016). The results presented in paper no. 4.4 suggests that iron chloride induces not only ROS production but also MDA production. MDA is the final product of lipid peroxidation. Investigation performed by He et al. (2008) shows, that Fe(III) directly interacts with nitrilotriacetate. Fe(III) binds with nitrilotriacetate (at a molar ratio of 1:1) and depresses the membrane stability of cells. Moreover, investigations performed by the same authors show that iron chloride raises alkaline phosphatase activity, SOD activity and GPx activity (He et al., 2008). In the study presented in paper no. 4.4 decreased activity of superoxide dismutase, catalase and glutathione peroxidase (GPx) was observed. SOD and CAT scavenge both intracellular and extracellular radicals. The antioxidant activity of these enzymes was insufficient to neutralize hydroxyl radicals generated by iron(III). Moreover, SOD and CAT cannot neutralize lipid hydroxyl-peroxide or lipid peroxidative products. Additionally, the activity of GPx was low and therefore it cannot neutralize these products.

As mentioned above, reactive oxygen species may interact with the nucleus and spindle apparatus. A statistically significant increase in DNA damage after iron chloride treatment in both cell lines was observed. The results obtained from both cell lines show that BALB/3T3 cells are more sensitive when compared to HepG2 cells after incubation with iron chloride. It means that iron chloride induces DNA damage-formation of single- and double-strand DNA breaks and AP sites. The micronucleus assay performed with the use of all concentrations shows statistically significant induction of

chromosomal aberrations after iron chloride treatment in both cell lines. Iron chloride evokes frameshift mutation and base–pair substitution mutation with similar frequency (paper no. 4.2). Moreover, in flow cytometric analysis of the cell cycle, a statistically significant dose-related decrease of the percentage of cells in the S and G2/M phases after Fe(III) exposure was observed. However, a statistically significant dose-related increase of the percentage of cells in subG1 and G0/G1 phases after exposure to iron chloride at concentrations range of 100-1000 μM in both cell lines was observed as well (paper no. 4.3). It can be concluded that iron chloride inhibits DNA proliferation. Moreover, iron affects ribonucleotide reductase activity, consequently inhibiting DNA synthesis. This blockage may be related to the deficiency of iron incorporation in ribonucleotide reductase, consequently inhibiting DNA synthesis. Moreover, it has been described that cyclin D-, cyclin E-, and cyclin A-associated kinase activity is inhibited after iron chelator exposure (Troadec et al., 2006). It can be concluded that trivalent iron arrests the cell cycle in G0/G1 phase. Moreover, aneuploidy peak (subG1) of DNA content was observed. This peak may represent cells that escaped mitotic arrest and replicated as multinuclear cells without dividing. Giant and multinuclear cells observed in microscopic analyses are the effect of this process (paper no. 4.2). What is more, apoptotic cells were observed. These effects are caused by reactive oxygen species and a weakened antioxidant enzyme cell system.

However, according to the research presented in work 4.5, apoptosis is induced not only in the intrinsic pathway related to mitochondrial damage and ROS production, which was supported by the determination of the concentration of caspases 3, 6 and 9. Similarly to the case of chromium chloride after incubation of cells with iron chloride, an increase in the concentration of caspase 12 was observed. It is activated in the process of apoptosis induced by damage to the endoplasmic reticulum. As in the case of chromium chloride, the formation of lipid droplets in the cytoplasm was observed, which was demonstrated using Raman and FT-IR spectroscopy. This means damage to the endoplasmic reticulum and induction of apoptosis in a pathway independent from mitochondria.

Moreover, a disturbance in the balance of Na^+ and K^+ ions was observed in the case of iron chloride. This disturbance of the resting membrane potential and ion transport across the cell membrane was observed. This leads to changes in membrane metabolism and its damage, which may induce apoptosis. These observations were made in papers 4.2 (LDH release assay) and 4.6. Moreover, an increase in Ca^{2+} concentration was observed in the cell, which indicates damage to RE and mitochondria, which was also found in papers 4.2, 4.3, 4.4 and 4.5. Additionally, there is the possibility of disturbing cell division by increasing the concentration of magnesium and the possibility of creating micronuclei (papers 4.2 and 4.3).

Nickel and its compounds are widely used in industry. There are many reports about nickel-induced DNA destruction. There are no reports about nickel cytotoxicity. In the study presented in paper no. 4.2, a decrease in cell viability was assessed by MTT and NRU and an increase in LDH release was observed. The results obtained from both cell lines show that HepG2 cells are more sensitive when compared to BALB/3T3 cells after incubation with nickel chloride. Investigations performed by Jia and Chen (2008) show that nickel induces ROS production. Moreover, induction of ROS production can decrease mitochondrial membrane potential (MMP) (paper no. 4.3). Moreover, nickel can inhibit mitochondrial succinate dehydrogenase activity leading to the loss of MTP (Wang et al., 2012). In the study presented in paper no. 4.4 nickel chloride induces ROS production, however, MDA production is on a smaller scale. Increasing concentration of intracellular ROS and MDA causes a decrease in SOD, CAT and GPx activities. It means that nickel(II) weakens cell defence mechanisms and plays the role of prooxidant. These observations confirm other authors' investigations (Kalaivani et al., 2014; Salnikow et al., 2000; Chen et al., 2003).

It has been proved that nickel increases the concentration of H3K4me3 and decreases the concentration of H3K9me3 histones. It can lead to DNA destruction and induce mutation (Chen i wsp., 2003; Arita i wsp., 2012; Hu i wsp.). In the study presented in paper no. 4.2, the increase in per cent of tail DNA in both cell lines was observed. The results obtained from both cell lines

show that BALB/3T3 cells are more sensitive when compared to the HepG2 cells after incubation with nickel chloride. Moreover, statistically significant induction of chromosomal aberrations assessed in micronucleus assay was observed. Microscopic analysis showed giant or multinuclear cells and cytoplasmic vacuolation. Additionally, the treated cells displayed characteristic apoptosis in comparison to control cells. The results obtained from both cell lines show that BALB/3T3 cells are more sensitive when compared to the HepG2 cells after incubation with nickel chloride. What is more, nickel chloride evokes frameshift mutation more often than base–pair substitution mutation. In my experiments, the incubation of both cell lines with nickel chloride caused an increase in the percentage of cells in the G2/M phase, while the percentage of cells in the S and G0/G1 phases decreased. Moreover, aneuploidy peak (subG1) DNA content was observed. The similar results were obtained by other authors (Lee et al., 2016; Chen et al., 2010; Shiao et al., 1998). It can be concluded that trivalent chromium arrests the cell cycle in the G2/M phase. The investigations conducted by Lee et al. (2016) suggest that nickel(II) induces G2/M cell accumulation by inhibiting the activity of cdk1 by directly stimulating the expression of p53 and $p21^{WAF1/CIP1}$.

Cell observations made in work 4.2 indicated the induction of apoptosis. Papers 4.3 and 4.4 showed that ROS over-production and inhibition of the antioxidant system are responsible for this process. The consequence of these changes is damage to mitochondria. The apoptosis process was also confirmed by the research carried out in paper 4.5 using Raman and FT-IR spectrometry. It was found not only an increase in the concentration of caspases 3, 6 and 9, which confirms the involvement of mitochondria in the induction of apoptosis but also an increase in the concentration of caspase 12 and the formation of lipid drops in the cytoplasm. These changes in cells confirm the possibility of inducing apoptosis as a result of damage to the endoplasmic reticulum. Moreover, an increase in the concentration of caspase 8, which is a marker of apoptosis induced by the extrinsic pathway, was observed.

Apoptosis was also induced by a mechanism dependent on the imbalance of elements found in the cell (paper 4.6). These include Na^+, K^+,

Mg^{2+} and Ca^{2+}. In the case of the first two, the functioning of the sodium-potassium pump is impaired, and as a consequence, resting membrane potential is lost. It leads to cell membrane damage and the formation of apoptotic bodies. Their presence was observed in the studies included in papers 4.2 and 4.6. However, the increase in the concentration of Ca^{2+} ions is caused by their release from RE, which is caused by damage to this organelle. Additionally, an increase in the concentration of Mg^{2+} ions causes disruption of the cell cycle, as stated in work 4.3.

The last of the analyzed elements was molybdenum. Molybdenum trioxide was non-toxic in both cell lines, assessed by MTT reduction assay and LDH release assay after molybdenum trioxide at the concentration range of 100-1400. In both cell lines, IC_{50} values could not be determined. In NRU assay, BALB/3T3 cells are more sensitive when compared to HepG2 cells after incubation with molybdenum trioxide (paper no. 4.2). Genotoxicity was assessed both by analyzing induction of micronuclei formation and DNA damage - formation of single- and double-strand breaks by comet assay in BALB/3T3 and HepG2 cells. In both assays increase in the percentage of tail DNA and the number of micronuclei in comet and micronucleus assays, respectively, were observed. The treated cells displayed characteristic apoptosis in comparison to control cells. The results obtained from both cell lines show that HepG2 cells are more sensitive when compared to BALB/3T3 cells after incubation with molybdenum trioxide. Molybdenum trioxide evokes frameshift mutation more often than base–pair substitution mutation (paper no. 4.2). The result of this activity is the destruction of DNA structure. DNA destruction should be removed by repair enzymes before replication. The study presented in paper no. 4.3 shows that DNA decreases in G0/G1 and S phases, while it increases in the G2/M phase after incubation with molybdenum trioxide at the concentration range of 100-1000 μM. It suggests that molybdenum activates the G2/M cell cycle checkpoint. G2/M DNA damage checkpoint serves to prevent cells from entering mitosis with genomic DNA damage. The similar results were obtained by Siddiqui et al. (2015). The same authors found that molybdenum induced intracellular ROS generation

in concentration dependent manner. These observations confirm my study (paper no. 4.4). The concentration of intracellular ROS and MDA increased after molybdenum treatment. However, the activity of SOD, CAT and GPx decreased. These observations confirm the study presented in paper no. 4.3 in which a decrease in MTP was observed. The decrease of MTP is induced by reactive oxygen species.

The synthesis of free radicals and weakening of the cell's antioxidant activity is one of the mechanisms leading to the induction of apoptosis. However, as in the case of the previously described compounds, in the case of incubation of cells with molybdenum trioxide, an increase in the concentration of caspases 3, 6, 9, and 8 was observed (paper 4.5). This confirms the possibility of activation of apoptosis as a result of damage to the mitochondria and, alternatively, the endoplasmic reticulum. This is confirmed by the results obtained by Ramaa and FT-IR spectroscopy. Another mechanism leading to apoptosis after the action of molybdenum trioxide was a disturbance of the balance of Na^+, K^+, Mg^{2+} and Ca^{2+} (paper 4.6). An imbalance in the concentration of Na^+ and K^+ ions cause a change in the structure and function of the cell membrane and the formation of apoptotic bodies. As one of the symptoms of apoptosis, they were observed in the results observed in papers 4.2 and 4.6. Other symptoms of apoptosis caused by Mg^{2+} and Ca^{2+} ion imbalance included: chromatin condensation, micronuclei formation (paper 4.2), mitochondrial damage (paper 4.3) and RE damage (paper 4.6).

Biologically active substances may interact with one another and modify the magnitude and nature of the toxic effect. Interactions may take place in the toxicokinetic phase and/or in toxicodynamic phase. There can be three types of interactions between medications: additive, synergistic, and antagonistic. An antagonistic effect occurs when the combined effect of two substances is less than the sum of each substance given alone. A synergistic effect occurs when the combined effect of two substances is greater than the sum of effects of each substance given alone. Potentiation, being a form of synergism, occurs when the toxicity of substance on a certain tissue is

enhanced when given together with another substance that does not have toxic effect on the same tissue (Fødevare Rapport).

On the basis of cytotoxicity assays the doses of the mentioned above microelements were chosen for interaction study. At 200 μM concentration in all used microelements no changes in cell viability were observed. Moreover, in some cases the cell viability was slightly stimulated (chromium chloride, iron chloride and nickel chloride). However, at concentration of 1000 μM and above that, decrease of cell viability was observed.

The following combinations were made:

Chromium chloride at concentration of 200 μM plus iron chloride at concentration of 1000 μM,
Chromium chloride at concentration of 200 μM plus nickel chloride at concentration of 1000 μM,
Chromium chloride at concentration of 200 μM plus molybdenum trioxide at concentration of 1000 μM,
Chromium chloride at concentration of 1000 μM plus iron chloride at concentration of 200 μM,
Chromium chloride at concentration of 1000 μM plus nickel chloride at concentration of 200 μM,
Chromium chloride at concentration of 1000 μM plus molybdenum trioxide at concentration of 200 μM.
All assays mentioned above were made.

Cr(III) and Mo(III) or Cr(III) and Ni(II) show antagonistic effect – chromium(III) protects from nickel(II) or molybdenum(III) toxicity. In cultures simultaneously treated with 200 μM of chromium chloride and 1000 μM of nickel chloride or molybdenum trioxide, an increase in cellular viability was observed when compared to control cells and cells incubated with nickel chloride or molybdenum trioxide at the concentration of 1000 μM. Moreover, the same observations were made in genotoxicity and mutagenicity assays. The number of micronuclei and percentage of tail DNA decrease when compared to control cells and cells incubated with nickel chloride or

molybdenum trioxide at the concentration of 1000 μM (paper no. 4.2). Moreover, the MTP increases when compared to control cells and cells incubated with nickel chloride or molybdenum trioxide at the concentration of 1000 μM. In cultures simultaneously treated with 200 μM of chromium chloride and 1000 μM of nickel chloride or molybdenum trioxide, a decreased percentage of cells in subG1 phase was observed. Moreover, the percentage of cells in G0/G1, S and G2/M phases is comparable with control cells (paper no. 4.3). These observations confirm studies conducted in paper no. 4.4. In cultures simultaneously treated with 200 μM of chromium chloride and 1000 μM of nickel chloride or molybdenum trioxide, a decrease of ROS and MDA concentration, while the increase of activity of SOD, CAT and GPx were observed. The study presented in paper no. 4.4 shows that chromium at concentrations of 100 and 200 μM increased superoxide dismutase and catalase activity. Moreover, the concentrations of ROS and MDA was low. However, nickel chloride and molybdenum trioxide at concentrations of 1000 μM induce oxidative stress while decreasing antioxidant enzymes activity. The similar results were obtained by Długosz et al. (2012) and Chen et al. (2018). It has been proved that chromium(III) at low concentrations statistically significantly increases superoxide dismutase and catalase activity. Probably this activity is the main mechanism responsible for the protective action of chromium(III) against nickel(II) or molybdenum(III).

Moreover, in the case of these elements, the dominant product of oxidative stress was intracellular ROS, while the concentration of MDA was low. Increased activity of SOD and CAT can neutralize intracellular ROS and protect from nickel(II) or molybdenum(III) toxicity. The increase in antioxidant enzymes activity leads to a decrease in ROS activity against mitochondria, cell membrane or DNA. The observed decrease, in higher concentrations of chromium(III), in catalase activity, is probably caused by the destruction of active site of catalase by the excess of Cr(III) (Chen et al., 2018). Moreover, at low concentrations chromium(III) increases DNA polymerase processivity and the catalytic activity and decreases the fidelity of DNA polymerase (Galaris and Evangelou, 2001, Snow and Xu, 1989). For this reason, single mutations were

observed in AMES assays after simultaneous incubation with chromium chloride at a concentration of 200 μM and nickel chloride or molybdenum trioxide at a concentration of 1000 μM.

The destruction of the active site of catalase by the excess of Cr(III), at higher concentrations, did not protect against nickel chloride or molybdenum trioxide at a concentration of 200 μM. For this reason in cells incubated with chromium chloride at a concentration of 1000 μM with nickel chloride or molybdenum trioxide at a concentration of 200 μM, the protective effect of chromium(III) was not observed. Moreover, an increase in ROS and MDA and a decrease in antioxidant enzyme activity were observed.

Cr(III) and Fe(III) show synergistic effects in cytotoxicity, genotoxicity and mutagenicity assays. In cultures simultaneously treated with 200 μM of chromium chloride and 1000 μM of iron chloride, an increase in the percentage of cells in subG1 and G0/G1 phases was observed when compared to control cells and cells incubated with chromium chloride and iron chloride. Moreover, the same mechanism was observed in the mitochondrial transmembrane potential assay. It has been reported, that chromium(III) and iron(III) can generate reactive oxygen species. However, the concentration of MDA after iron(III) treatment was higher when compared to intracellular ROS. MDA is the final product of lipid peroxidation. The antioxidant activity of these enzymes was insufficient to neutralize hydroxyl radicals generated by both chromium(III) and iron(III). Moreover, SOD and CAT cannot neutralize lipid hydroxyl-peroxide or lipid peroxidative products. Additionally, the activity of GPx was low and insufficient to neutralize these products. Moreover, products of lipid peroxidation attack biomolecules and organelles: membrane phospholipids, DNA and mitochondria (Kohgo et al. 2008). What is more, Cr(III) interacts with both base and phosphate groups of DNA, and Fe(III) interacts with DNA bases (Moriwaki et al., 2008). These independent mechanisms can cause synergistic effects.

Chromium and iron were also tested for their ability to inhibit the replication of DNA and RNA viruses (paper 4.1). It has been shown that chromium chloride and iron chloride at concentrations of 200, 400, 600, 800,

1000 μM, when used alone, inhibit the multiplication of the HSV-1 and BVDV viruses. Based on the results obtained, the concentrations of elements were selected for interaction testing. Microelements used simultaneously in the following interactions: 1) chromium chloride at a concentration of 200 μM plus iron chloride at a concentration of 1000 μM, 2) chromium chloride at a concentration of 1000 μM plus iron chloride at a concentration of 200 μM, 3) chromium chloride at a concentration of 400 μM plus ferric chloride at a concentration of 800 μM and 4) chromium chloride at a concentration of 800 μM plus ferric chloride at a concentration of 400 μM act synergistically in reducing the DNA copy number of both viruses used. The probable mechanism is based on the possible interaction of microelements with DNA and RNA of viruses. Both elements have the ability to interact with nucleic acids, causing their damage.

Conclusions

The analysis of the obtained results brought the following conclusions, the most important of which are presented below.

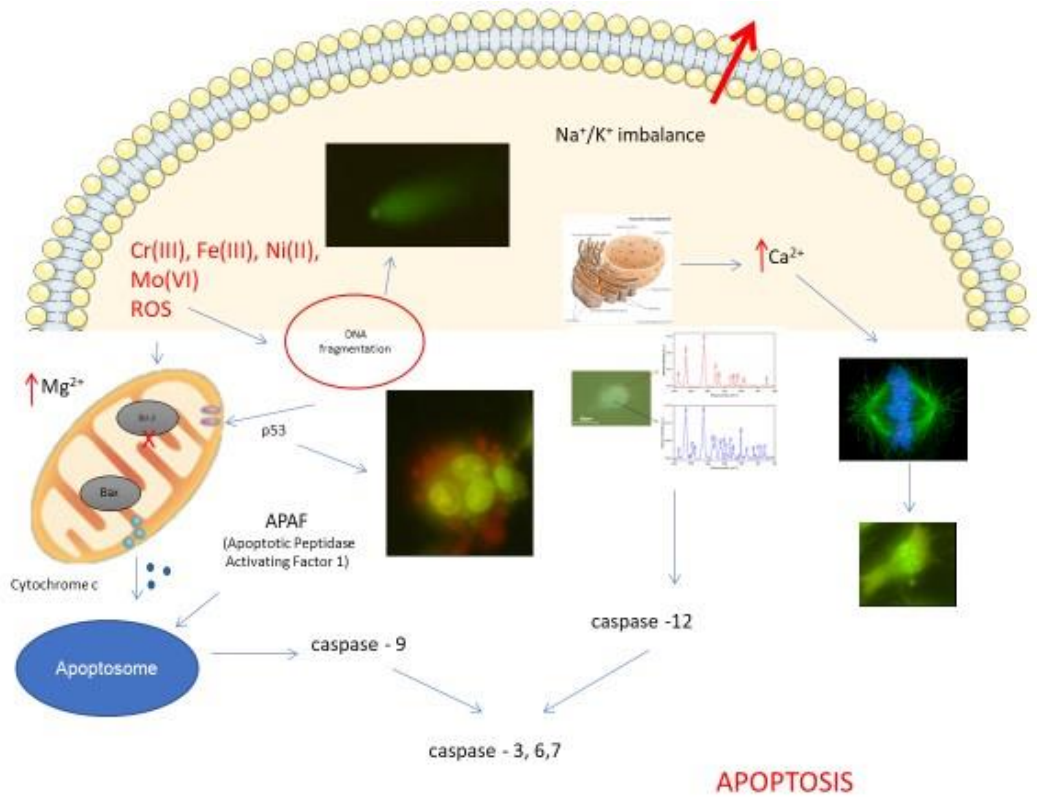
1. Cell membrane may be the first organelle in which the effect of chromium chloride is observed. The damage of mitochondria and then lysosomes follows cell membrane damage in both cell lines. Mitochondria of cancer cells are more sensitive when compared to mitochondria of normal cells after iron chloride treatment.
2. Chromium chloride, iron chloride, nickel chloride and molybdenum trioxide induce DNA damage: single- and double-strand breaks and AP sites.
3. Chromium chloride molybdenum trioxide and nickel chloride evoke frameshift mutation more often than the base–pair substitution mutation. Iron chloride evokes frameshift mutation and base–pair substitution mutation with similar frequency

4. Chromium chloride iron chloride, nickel chloride and molybdenum trioxide cause functional disorders of the spindle apparatus, leading to karyokinesis without cytokinesis.
5. Chromium chloride, iron chloride, nickel chloride and molybdenum trioxide induce reactive oxygen species production. Iron chloride causes lipid peroxidation.
6. The increase of activity of SOD and CAT after chromium chloride treatment at low concentrations was observed. However, at higher concentrations, decreased activity of this enzyme was observed. Iron chloride, nickel chloride and molybdenum trioxide inhibit antioxidant activity of SOD, CAT and GPx.
7. Chromium chloride nickel chloride and molybdenum trioxide arrest cell cycle in G2/M phase. Iron chloride arrests the cell cycle in G0/G1 phase.
8. Chromium chloride, iron chloride, nickel chloride, and molybdenum trioxide induce apoptosis in the mitochondrial, endoplasmic reticulum and ion imbalance-dependent intrinsic pathway. Nickel chloride induces apoptosis in the extrinsic pathway.
9. Chromium chloride and iron chloride show synergistic effects.
10. Chromium chloride and molybdenum trioxide or chromium chloride and nickel chloride show antagonistic effects – chromium chloride protects from nickel chloride or molybdenum trioxide toxicity.

Summary

The aim of the research presented in these studies was to investigate the metabolism of chromium(III), iron(III), nickel(II) and molybdenum(III). In addition, the nature of the interaction between chromium(III) and other elements was investigated. This is a very important problem in modern medicine. In recent years, there has been an increase in the intake of vitamin and mineral supplements and dietary supplements. Biomaterials containing these micronutrients are also used on a wide scale in medicine. In human and animal organisms there may be exceeded safe concentrations of these elements leading to unknown interactions. That is why the obtained results are very important. These are the first reports in the literature concerning the

interaction between chromium chloride and iron chloride or nickel chloride or molybdenum trioxide. That is why, I have not been able to discuss the problem with other scientists. Investigations carried out demonstrated the possibility of apoptosis induction in cells via an extrinsic (nickel) and intrinsic (chromium, nickel, iron and molybdenum) pathway. The available literature pointed to the involvement of ROS in the induction of apoptosis. The present study showed that not only ROS are responsible for the induction of apoptosis, but also the impairment of antioxidant enzymes. ROS affect mitochondria, causing mitochondrial damage, release of cytochrome *c* and activation of caspases: 9, 3, 6 and 7. In addition, an increase in Mg^{2+} ion concentration causes a decrease in Bcl-2 expression and an increase in Bax expression, which affects cytochrome *c* release. In addition, apoptosis is induced by damage to RE, which leads to the release of Ca^{2+} ions into the cytoplasm. The excess of these ions negatively affects cell cycle regulation and its inhibition in the G1/S and G2/M phases and during division spindle formation. Damage to RE also causes the formation of lipid droplets in the cytoplasm and activation of caspase 12 leading to apoptosis. In addition, an imbalance of Na^{+}/K^{+} ions leads to disruption of the resting cell membrane potential and vesicle formation on the membrane surface. Regardless of their ability to induce apoptosis by altering the metabolism of the cell membrane, mitochondria and RE, the elements themselves and through oxygen free radicals interact with the cell's DNA to cause damage and mutation.



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5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

In vitro and in vivo study of elemental interactions

In 2000 I completed studies at the Faculty of Biology and Geo-Sciences, Maria Curie-Sklodowska University in Lublin. The title of my thesis was *Spontaneous and induced production of cytokines by bone marrow stroma in patients with multiple myeloma and acute myeloid leukaemia*. In the same year, I started my work as an assistant at the Department of Cell Biology, the Faculty of Mathematics and Natural Sciences of the Catholic University of Lublin. The main aims of my work were studies in the metabolism of microelements and the use of alternative methods in toxicity tests. The first investigated microelement was trivalent chromium. The mechanism of chromium(III) was then unknown. Moreover, some scientists suggest that chromium(III) is involved in insulin receptor activation and metabolism of fats. Chromium(III) has been recommended, as a fat tissue reducer. The other microelement was vanadium(V). Additionally, the mechanism of their

interaction was unknown. I was also involved in studying the interactions of selenium(IV) and zinc(II) with vanadium.

In rats which received in drinking water chromium chloride displacement of osmotic fragility curves were observed. In chromium(III) treated rats, an increase of minimal, maximal and mean osmotic fragility of RBC was observed. Erythrocytes were also examined using scanning electron microscopy. The morphological changes in erythrocytes were observed. Erythrocytes isolated from chromium-treated rats had characteristic membrane invaginations. Moreover, an increase of MDA concentration in chromium-treated rats was observed. These observations suggest that chromium(III) may act as a pro-oxidant.

The results of the research have been published in the papers and presented at scientific conferences: A1, A2, A3, A4, R1, R2, P1, P2, P3, P4, P5.

In 2006 I began cooperating with the Department of Microbiology and Clinical Immunology at the Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn. Supervised by the Head of the Department, prof. dr hab. Andrzej K. Siwicki, I wrote the doctoral thesis. In 2011, I started work at the Institute of Environmental Engineering. In the team I created, I continued my research on chromium(III) and iron(III) interactions. *In vivo* investigations have shown that chromium(III) and iron(III) decrease IL-1 concentration, while no differences in IL-6 concentration and the proliferative response of lymphocytes and the metabolic activity of phagocyting cells were observed. Similar results have been observed in lysozyme and gamma-globulins levels. Moreover, synergistic interaction between chromium(III) and iron(III) was observed. Chromium chloride when intraperitoneally injected in mice causes displacement of osmotic fragility curves. In chromium(III) and iron(III) treated mice, an increase of minimal, maximal and mean osmotic fragility of RBC was observed. Simultaneous treatment with chromium(III) and iron(III) suggests the synergistic interaction between these elements and the intensification of hemolytic activity of these elements. Iron serum

concentration is lower in mice exposed to chromium(III). TIBC and ITS were also reduced following chromium(III) administration. Simultaneous injection of iron(III) and chromium(III) decreased statistically significant iron and TIBC concentration and percentage of ITS. Additionally, *in vitro* investigations were performed. Chromium(III) and Iron(III) caused a decrease in fibroblast viability an increase in IL-1 secretion, and a decrease in IL-6 *in vitro*. In addition, chromium(III) exerts protective functions against iron(III)-induced cytotoxicity in cells of the BALB/c 3T3 line when simultaneously incubated with both elements. However, preincubation of BALB/c 3T3 line cells with Fe(III) protects against Cr(III)-induced toxicity.

The results of the research have been published in the papers A5, A6, A7, A8, A10, A13, A21, P6, P7, P11, P12, P13, P14, P15, P16.

After obtaining my PhD, in 2010, I was employed at the newly established Institute of Environmental Engineering. In 2011, I became head of the Environmental Biology Laboratory. In the newly established research team, I continued the research on micronutrient interactions started in my PhD thesis. However, the newly established unit did not have laboratory equipment to conduct research, so I continued my research at the University of Warmia and Mazury in Olsztyn.

In 2012, I completed a research scholarship at the Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury. As part of this scholarship, the investigation activity of chromium(III), iron(III) and nickel(II) were conducted. The results of the study on the interaction of chromium and iron on antiviral activity are presented in section 4 of this document.
The results have been published in papers and presented at scientific conferences: A12, R6, P18, P22.

In 2014, I completed a research scholarship at the Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury. During my scholarship, I carried out studies on the

interaction of micronutrients, i.e. chromium with nickel, iron and molybdenum. These include the results already described in section 4 of this document.

The research results have been published in papers and presented at scientific conferences: A18, A19, A22, R7, R8, R14, R15, R17, R20, P23

In addition, I have studied the interactions of iron with nickel and iron with molybdenum. I have also undertaken studies on the interactions of molybdenum with chromium or nickel or cobalt or iron.

The decrease in cell viability was observed after incubating BALB/3T3 and HepG2 cells with iron chloride or nickel chloride in all used assays: MTT reduction assay, LDH release assay and neutral red uptake assay. The increased DNA damage in both used microelements in both cell lines was observed. The micronucleus assay performed with the use of all concentrations showed induction of chromosomal aberrations in both tested microelements in both cell lines. Nickel chloride evokes frameshift mutation more often than base–pair substitution mutation. However, in the case of iron chloride the frequency in frameshift and base–pair substitution mutation was similar.

Additions of Fe(III) at a concentration of 200 μM plus Ni(II) at a concentration of 1,000 μM or Fe(III) at a concentration of 1,000 μM plus Ni(II) at a concentration of 200 μM showed a synergistic effect in a decrease in cell viability assessed by LDH, and NRU assays. However, in MTT assay, iron(III) at a concentration of 200 μM protects from nickel(II) toxicity at a concentration of 1,000 μM. Iron is critical for the formation of iron-sulphur clusters in mitochondrial succinate dehydrogenase. In low doses, iron probably provides proper activity of the above-mentioned enzyme and protects from nickel toxicity. Similar results were observed in genotoxicity and mutagenicity assays. Iron(III) at a concentration of 200 μM protects from nickel(II) toxicity at a concentration of 1,000 μM.

The next analyzed interaction was that of iron chloride plus molybdenum trioxide. BALB/3T3 and HepG2 cells displayed statistically

significant decreases in cell viability after molybdenum trioxide at a concentration range of 400-1400 μM treatment, as assessed by MTT assay. In the case of molybdenum trioxide, toxic effects were not observed in the LDH assay. Similar effects were observed after molybdenum trioxide treatment in HepG2 cells in NRU assay. However, iron chloride at a concentration range of 400-1400 μM was toxic in all the mentioned above tests. Moreover, both microelements increase the percentage of tail DNA, number of micronuclei and number of mutations. Molybdenum trioxide evokes base–pair substitution mutation more often than frameshift mutation. However, in the case of iron chloride, the frequency in frameshift and base–pair substitution mutation was similar. The mixture of iron chloride at the concentration of 200 μM and molybdenum trioxide at the concentration of 1,000 μM in the first case, and - in the other case – the mixture of iron chloride at the concentration of 1,000 μM and molybdenum trioxide at the concentration of 200 μM caused synergistic effects which were observed in the decreased viability, increased percentage of tail DNA, number of micronuclei, number of mutations, ROS, MDA and decrease of SOD, CAT i GPx activity

The results of these studies have been published in papers: A16, A17, A20, A23, R9, R10, R11, R12, R13, R21, P19

Research on micronutrient metabolism also continued with further internships. In 2020, I completed a three-month research internship at the Institute of Nuclear Physics of the Polish Academy of Sciences. As part of this collaboration, I continued research on the induction of apoptosis in cells under the influence of chromium chloride, iron chloride, nickel chloride and molybdenum trioxide using Raman and FT-IR spectrometry. The results of these studies are described in section 4 of this document.

The results of the study were published in the paper: A28.

In 2019 2021, and 2023, I completed three scholarships at the Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University in Toruń. The results of these studies are described in section 4 of this document.

The results of the study were published in the paper: A29

Investigations of cytotoxicity and genotoxicity of Inosine pranobex

I complted another scholarship at the Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury from 1 III to 7 IV 2014. Inosine pranobex, a combination of the p-acetamidobenzoate salt of N,N-dimethylamino-2-propanol and inosine in a 3:1 molar ratio, is an immunomodulating anti-viral drug in humans. This study was supported by the National Centre for Research and Developments, grant title: *Research on innovative drug with an immunostimulating effect in animals* (POIG.01.03.01-28-108/12). The aim of this study was to investigate cytotoxicity, genotoxicity and mutagenicity of Inosine pranobex in normal and cancer cells. The results obtained from MTT reduction, LDH release, NRU, comet and micronucleus assays in both cell lines show that HepG2 cells are more sensitive when compared to BALB/3T3 cells.

The results of the research have been published in the papers: A14, A15.

Investigations the biological properties of biomaterials

In 2021, I started working at the Department of Surgical Medicine with the Medical Genetics Laboratory, Institute of Medical Sciences, Collegium Medicum, Jan Kochanowski University in Kielce. At my new workplace, I began research into the application of new biomaterials in medicine. To this end, I have established cooperation with the Kielce University of Technology, the Warsaw University of Technology and the Łukasiewicz-Lodz Institute of Technology. As part of this collaboration, I am investigating the biological properties of newly synthesized nanostructures that can be used in anticancer

therapy. One such structure is nanosilver ions deposited on a carbon matrix. The toxicity of silver nanostructures has been investigated in many scientific studies. They showed the danger of the above-mentioned structures inducing cytotoxic and especially genotoxic and mutagenic effects. Hence, an attempt was made to synthesise new structures that would eliminate these effects. The work involved the development of a method for the synthesis of new nanostructures. In turn, I was responsible for testing its biological properties. Tests were carried out using the L929 line and the studies showed the absence of genotoxicity and mutagenicity that was often observed with silver nanostructures.

The results of the research were published in papers and presented at scientific conferences A26, R19.

The collaboration continues and research are conducted into the application of the developed nanostructures in anti-cancer therapy. In addition, I am researching the development of new materials that can serve as a matrix for culturing primary pancreatic cancer cells in 3D. This research is being conducted in collaboration with the Lukasiewicz-Lodz Institute of Technology in Lodz. As part of this collaboration, an application for funding from the National Science Centre under the OPUS programme was prepared.

Research results have been presented at scientific conferences P16

I have also researched modern biomaterials for use in medicine in collaboration with the Department of Biochemistry and Biotechnology, Faculty of Pharmacy, Medical University of Lublin. In turn, I was responsible for testing the genotoxicity of the biomaterial. The result of this collaboration is a paper entitled: *Comprehensive biological assessment of biomimetic curdlan-based osteochondral scaffold - In vitro, ex vivo, and in vivo studies in accordance with 3Rs principle*, K. Klimek, S. Terpilowska, A. Michalak, R. Bernacki, A.

Nurzynska, M. Cucchiarini, M. Tarczynska, K. Gaweda, S. Głuszek, G. Ginalska, which will be send to the journal.

Study of selected parameters of patients with brain injury

In 2019, I started a collaboration with the Medical University of Lublin, 1st Department of Anaesthesiology and Intensive Care, SPSK4 in Lublin concerning the study of selected parameters of patients with brain damage. In addition, the influence of the brain-gut axis on parameters of patients hospitalised in intensive care units. This collaboration continues to this day. As a result of this collaboration, I studied the concentrations of caspases and selected pro- and anti-apoptotic proteins in patients with brain damage. As part of this research, I was responsible for the determination of caspase concentrations: 3, 6, 8, 9 and 12. In addition, I determined the concentrations of Caspase Activated DNase (CAD), Tumour protein p53 (TP53), B-Cell Leukaemia/Lymphoma 2 (Bcl-2), Bcl2 Associated X Protein (BAX), p53 Upregulated Modulator of Apoptosis (PUMA), Apoptotic Peptidase Activating Factor 1 (APAF1) and Bcl2 Associated Death Promoter (BAD). All the above-mentioned parameters were determined in bronchoalveolar lavage fluid (BALF) from patients with isolated traumatic brain injury (TBI) admitted to the Department of Anaesthesiology and Intensive Care, SPSK4 in Lublin. Both caspases and pro- and anti-apoptotic proteins are indicators of the apoptosis process of airway epithelial cells. Hence, the aim of this study was to determine the likelihood of apoptosis of airway lining cells, which may significantly affect respiratory fitness. This is important as these patients are subjected to intensive mechanical ventilation, which promotes pulmonary complications, i.e. respiratory failure, pneumonia, acute lung injury and respiratory distress syndrome (ALI/ARDS). The study showed higher levels of caspase-3, caspase-6 and caspase-8, in patients who died before the 28th day of ICU stay. Furthermore, the levels of all labelled caspases in the BALF of patients with isolated severe brain injury were shown to be associated with injury severity and 28-day mortality. This suggests induction of apoptosis, both in the extrinsic pathway (increase in caspase-8) and intrinsic pathway (increase in

caspase-9). In addition, the increase in caspases suggests apoptosis induced by mitochondrial dysfunction (increase in caspases-3, 6 and 9) as well as endoplasmic reticulum (increase in caspase-12). In the next step, the concentrations of selected pro- and anti-apoptotic factors mentioned above were examined. Patients showed an increase in the proteins observed during apoptosis. Research is continuing and includes the effect of oxygen therapy in these patients on the induction of oxidative stress, which may be responsible for the induction of apoptosis.

The findings have been published in papers and presented at scientific conferences A24, A25, A27, P27. This collaboration also resulted in another paper entitled: Multiorgan microbiome and brain axis as a potential therapeutic target in TBI, D. Siwicka-Gieroba, C. Robba, R. Badenes, K. Kotfis, S. Terpilowska, M. Bielacz, W. Dabrowski, which will be send to journal.

Other current research

Within the cooperation with Prof. Andrzej Jaroszyński, MD, PhD, from the Department of Internal Medicine and Family Medicine, Collegium Medicum, Jan Kochanowski University in Kielce, I performed a number of determinations of parameters in the blood plasma of dialysis patients. These were: caspase 1, caspase 2, caspase 3, tumour necrosis factor receptor superfamily member 1A (TNFRSF1A), tumour necrosis factor receptor superfamily member 1B (TNFRSF1B), tumour necrosis factor receptor superfamily member 10B (TNFRSF10B), S100 Binding Protein, heat shock protein family B (HSPB1), Myelin basic protein (MBP), Glial fibrillary acidic protein (GFAP), Microtubule-associated protein tau (MAPt), IL1RL1, IL-6, MDA-modified LDL (ox-LDL/MDA), Antioxidative Capacity (ImAnOx (TAS/TAC)), endothelin 1 (EDN1), Noradrenaline (NE), Asymmetric dimethylarginine (ADMA) and miRNAs: miRNA-21-5p, miRNA-93-5p, miRNA-122-5p, miRNA-132-3p, miRNA-142-3p, miRNA-195-5p, miRNA-625-5p. The results of these studies are currently being analysed and manuscripts are being prepared.

Other achievements (the results of the research have been published in the papers: A9, A11, RM1, RM2, RM3, RM4, RM5, RM6, RM7, RM8, RM9, RM10, RM11, RM12, RM13, RM14, RM15, RM16, RM17, RM18, RM19, R3, R4, R18, P8, P19, P10, P17, P20, P21, P24, P25, P26, M1, M2).

In addition to a series of studies on the interactions of elements, I have published a number of papers about the metabolism of various elements, vitamins, as well as about the latest achievements in medicine and modern substances used in it. These include functional foods, nanomaterials and biomaterials.

The result of my activity are two monographs (M1, M2), in which, extensive information about the basics of cell biology or microbiology was collected, as well as, practical aspects of work in a microbiological laboratory.

6. Presentation of teaching and organizational achievements as well as achievements in the popularization of science or art

a) Assistant supervisor in the doctoral thesis of Katarzyna Czarnek, M.Sc., since 2018, at the Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn. Doctoral thesis defence 24 September 2019.

b) Achievements in teaching and in the popularisation of science

Jan Kochanowski University in Kielce

- conducting classes for medical students 2021-present

Physiology with cytophysiology (lecture, classes, laboratories) Polish students

Physiology with cytophysiology (lecture, classes, laboratories) English division

Pathophysiology (classes) English Division

Haemostasis and Thrombosis (lecture) English division

Basis in cell biology (lecture) English division

In vitro studies in cosmetology (lecture, classes, laboratories)

- supervisor of studies in the field of medical student English division, 2023-now

The John Paul II Catholic University of Lublin

- development of original curricula for students of Environmental Engineering in the following subjects, 2010- 2020:

Biology and Ecology (lecture and laboratories)

Environmental biology (lecture and labs)

General microbiology with technical elements (lecture and laboratories)

Microbiological water pollution assessment (lecture and laboratories)

Environmental toxicology (lecture and laboratories)

Dewatering and irrigation (lecture and laboratories)

Sewage sludge treatment processes (lecture and laboratories)

Environmental protection (lecture and labs)

Protection of intellectual property (lecture and laboratory)

- development of original curricula for environmental engineering students for monograph lectures, 2010- 2020:

Biotechnology in environmental engineering

Microbiology, hygiene, environment- safety and quality in industrial practice

- teaching Engineering Seminar and Engineering Laboratory, 2011-2020

- To conduct lectures and exercises in the following subjects for Environmental Engineering students.

Biology and ecology (lecture and laboratories)

Environmental biology (lecture and laboratories)

General microbiology with technical elements (lecture and laboratories)

Microbiological water pollution assessment (lecture and laboratories)

Environmental toxicology (lecture and laboratories)

Dewatering and irrigation (lecture and laboratories)

Sewage sludge treatment processes (lecture and laboratories)

Environmental protection (lecture and labs)
Protection of intellectual property
Biotechnology in environmental engineering
Microbiology, hygiene, environment - safety and quality in industrial practice
- Promoter of 48 engineering theses, 2012-2021
- Reviewer of 23 engineering theses, 2013-2017
- supervisor of studies in the field of Environmental Engineering, 2011-2016
- Project coordinator of VI and VII Lublin Science Festival in 2009, 2010
- Project coordinator of the Faculty of Social Sciences in the 9th Lublin Science Festival and Science and Technology Days, 2012.
- presentations in the 9th Lublin Science Festival and Science and Technology Days, 2012
Sylwia Terpiłowska, Joanna Dyrda-Muskus, Tomasz Pajor, Academy of Science
Sylwia Terpiłowska, Aleksandra Ptaszek, Robert Stachyra, Monika Szelest, Katarzyna Czarnek How does activated sludge 'work'?
Sylwia Terpiłowska, Mariusz Piasecki, Adam Wszołek, Waldemar Lakan, Tomasz Bednarowicz, Aleksandra Bułaś, Modern company caring for the environment
Sylwia Terpiłowska, Bacteria - enemies or allies?
Katarzyna Czarnek, Monika Szelest, Robert Stachyra, Sylwia Terpiłowska, Do you want to build your own cell models?
Sylwia Terpiłowska, In vitro and/or in vivo? - or a few words about testing new substances ...
Monika Szelest, Robert Stachyra, Katarzyna Czarnek, Sylwia Terpiłowska, How to see what is invisible to the naked eye?
Robert Stachyra, Katarzyna Czarnek, Monika Szelest, Sylwia Terpiłowska, Microscopic allies, or how organisms invisible to the naked eye clean our wastewater

- Lecture entitled "How much and how...? or dietary titbits, 2012, BIOACTIVES Scientific Association, Faculty of Chemistry UMSC and the University of the Third Age in Stalowa Wola

c) Organisational achievements

- a member of the working team responsible for applying of funds for clinical studies, 2023 -now

- manager of Environmental Biology Laboratory, Institute of Environmental Engineering, Faculty of Catholic University of Lublin in Stalowa Wola, 2011-2019. Organisation of work in the Laboratory, purchase of equipment, development and supervision of teaching and research activities. Development of the basis of the GLP system in the Laboratory.

- Member of the Council of the Institute of Environmental Engineering, 2011-2019.

- Representative of the Institute of Environmental Engineering in the Faculty Education Quality Committee, Faculty Coordinator of the Internal Education Quality Assurance System, 2011- 2016. Development of the Faculty Education Quality Assurance System at the Faculty of Law and Social Sciences of the John Paul II Catholic University of Lublin in Stalowa Wola. Development of the Faculty Educational Quality Assurance System and supervision of its implementation.

- Member of the Faculty Commission for the distribution of funds for scientific research, contacts with the KBN and the Research and Scientific Projects Service of the Catholic University of Lublin 2011-2015. Development of criteria for the distribution of funds from the statutory grant and the distribution of these funds among research topics.

- Chairperson of the Curriculum Committee for the development of the curriculum for the faculties of Environmental Engineering and Materials Science, 2011-2015. Preparation of the curriculum for the above faculties.
- Implementing purchases of laboratory equipment and reagents at the Faculty of Overseas Social Sciences in Stalowa Wola, 2012. Training of the Faculty staff in the field of Public Procurement Law, preparation of the public procurement plan. Preparation of tender documentation in accordance with the guidelines of the Catholic University of Lublin and universally applicable acts of law.

- Member of the Team for elaboration of development strategy of the Catholic University of Lublin until 2020, 2012. Within the working sub-task force entitled Administration and Finance Area, I was responsible for preparation of SWOT analysis, development of strategic and operational goals and strategies in this area. Development of an overall strategy for the development of the University.

- Member of the Senate Administration and Finance Committee, 2012-2016.
The tasks of the Commission, in whose work I took an active part, included in particular:
1) overseeing the management of the premises of the Catholic University;
2) Issuing opinions on applications for benefits from the Social Benefits Fund of the Catholic University of Lublin, in accordance with the rules laid down in the regulations of the Social Benefits Fund;
3) Issuing opinions on applications for the cancellation and extension of the repayment period of loans granted from the Welfare Fund of the Catholic University of Lublin (KUL)
- member of the Senate Committee on Quality of Education, 2012-2016.
The tasks of the Commission, whose work I took an active part in, included in particular:
1) coordinating quality assurance policies and procedures;

2) introducing and improving procedures for European standards for internal quality assurance in higher education institutions;
3) overseeing the proper conduct of the educational process;
4) monitoring and periodically reviewing programmes and their outcomes;
5) monitoring the criteria and procedures for the assessment of students;
6) verifying the staffing of courses and teaching loads of academic staff
7) initiating the environmental accreditation procedure;
8) recommending new forms and directions of education to the Senate of the Catholic University of Lublin;
9) supervising the process of monitoring the professional fate of graduates;
10) coordinating cooperation with employers, labour market representatives and other entities to ensure and develop the implemented pro-quality activities.

- Member of the Commission to carry out an internal audit on management at the Catholic University of Lublin and compliance with internal and external regulations by University employees, 2012-2016
The purpose of the Commission's work was to carry out an audit of governance at the Catholic University of America and compliance with internal and external regulations by University employees, in particular:
1) to identify problems and identify remedial measures for the implementation of organisational governance at KUL,
2) assessing the efficiency and effectiveness of the activities of the organisational units at KUL,
3) recommending to the Rector actions and solutions to improve the management system of the University.

- Member of the Committee for updating the application for funding of the project entitled "Teaching and research facilities necessary for the establishment and development of the environmental engineering faculty at the Faculty of Overseas Studies of the Catholic University of Lublin in Stalowa

Wola", 2014. The aim of the team was to update the application for funding of the Construction and equipping of the Environmental Engineering building.

- Chairperson of the working team for the work on responding to the Implementing Authority on the deployment of equipment and furnishings in the building of the Institute of Environmental Engineering and the reconciliation of this deployment with the Institute's development and teaching plan, 2014. Development of scientific, teaching development plans, development of equipment layout plan for the Environmental Engineering building.

- Coordinator for the Rector's scholarship for the best students and the best doctoral students at WZPiNoS, 2015-2016. Opinion on applications for the Rector's scholarship for the best students. Assessment of students' academic achievements and determination of scores.

- Member of the working team supporting the team for servicing the project entitled "Teaching and research facilities necessary for the establishment and development of an environmental engineering faculty at the Faculty of Overseas Social Sciences of the Catholic University of Lublin in Stalowa Wola", 2015.

- member of the Tender Committee for the purchase of equipment for the Environmental Biology Laboratory, 2015. development of descriptions of the subject of the contract, selection of the most advantageous offer.

- Chairperson of the Tender Committee for the tender entitled: Supply of laboratory equipment for the needs of the Environmental Biology Laboratory of the John Paul II Catholic University of Lublin in Stalowa Wola, 2018. Development of descriptions of the subject of the tender, selection of the most advantageous offer.

7. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.

International and national awards for scientific or artistic activities

1st Team Award of the Second Degree of the Rector of the Catholic University of Lublin for creative activity in administration and special organisational achievements, 2007

3rd Degree Team Award of the Rector of the Catholic University of Lublin for outstanding performance as the coordinator of the 6th Lublin Science Festival, 200

1st Degree Team Award of the Rector of the Catholic University of Lublin for exemplary performance as faculty coordinator of the Lublin Science Festival, 2011

3rd Degree Team Award of the Rector of the Catholic University of Lublin for an outstanding project for the 8th Lublin Science Festival, 2011

bronze medal for long service, President of the Republic, ID number 308-2013-145, 2013

individual award of 3rd degree of the Rector of the Catholic University of Lublin for original and creative scientific achievements and outstanding organisational and social activity and popularisation of science, 2013

Signature Not Verified
Dokument podpisany przez Sylwia Terpiłowska
Data: 2024.01.25 18:59:32 CET

(Applicant's signature)