F O U R T H W O R K S H O P

ON BIOLOGICAL ACTIVITY OF METALS AND METAL COMPOUNDS

PROGRAM AND ABSTRACTS

NOVEMBER 24-25, 2009

SOFIA, BULGARIA

THE WORKSHOP IS ORGANIZED
BY THE INSTITUTE OF EXPERIMENTAL PATOLOGY AND PARASITOLOGY
UNDER THE AUSPICES OF
THE BULGARIAN ACADEMY OF SCIENCES

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THE PROGRAM OF THE WORKSHOP

Thuesday, November 24th

10.00 h-10.15 h ***OPENING REMARKS***

Session A: ROLE OF METALS IN NORMAL AND PATHOLOGICAL PROCESSES

Chairpersons:

Assoc. Prof. Yana Mizinska, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

Assoc. Prof. Reni Kalfin, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Assist. Prof. Yordanka Gluhcheva, PhD
Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences

10.15-10.45 h ABOUT IRON, PEOPLE AND LIFE

R. Alexandrova, R. Kalfin, O. Alexandrov

10.45-11.00 h THE AFFECT OF COBALT SALTS ON SOME WEIGHT INDICES IN DEVELOPING MICE

Y. Gluhcheva, M. Madzharova, V. Atanasov, R. Nizamova, M. Mitewa, E. Pavlova

11.00-11.20 h COFFEE BREAK

11.20-11.50 h LET US REMEMBER ZINC

R. Alexandrova, M. Georgieva, T. Todorova

11.50-12.05 h MAGNESIUM AND ZINC

M. Gursov, T. Ivanova

12.05-12.20 h EFFECTS OF METALS ON BONE MINERALIZATION

Zh. Ivanova, P. Genova-Kalou, A. Toshev

12.20 -13.30 h LUNCH TIME

13.30-13.45 h PRESENTATION OF THE FIRM LABSYSTEMS Ltd.

G. Gruev

13.45-14.20 h APPARATUSES OF FIRM SARTORIUS STEDIM BIOTECH IN THE FIELD OF CELL CULTIVATION

Y. Mihov

14.20-14.45 h **COFFEE BREAK**

Session B: METAL COMPOUNDS AS ANTIMICROBIAL AGENTS

Chairpersons:

Prof. Radka Argirova, MD, PhD, DSc
National Centre for Infectious and Parasitic Diseases

Prof. Mariana Mitewa, PhD, DSc
Faculty of Chemistry, Sofia University "St. Kliment Ohridski"

Assist. Prof. Radostina Alexandrova
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

14.45-15.00 h **MICROBIOLOGICAL ACTIVITY OF THE POLYETHER IONOPHORE MONENSIC ACID AND ITS METAL COMPLEXES**

R. Zhorova, I. N. Pantcheva, M. Mitewa

15.00-15.15 h **CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF RARE-EARTH METAL COMPLEXES WITH HYDROXYCOUMARINS IN CELL CULTURES**

P. Genova- Kalou, S. Raleva, A. Hinkov, I. Manolov, S. Gurkova, R. Argirova

15.15-15.30 h **MELOXICAM AND METAL COMPLEXES OF MELOXICAM AS POTENTIAL ANTITUMOR AGENTS**

R. Alexandrova, Ts. Gencheva, I. Ivanova, K. Timcheva, G. Marinescu, D. Culita, L. Patron

15.30-15.45 h **COPPER CHELATION THERAPY AND CANCER TREATMENT**

R. Alexandrova, K. Timcheva, S. Velev

15.45-16.00 h **GENETIC POLYMORPHISMS OF GLUTATHIONE S-TRANSFERASES PI AND OMEGA IN RESPONSE TO CHEMOTHERAPY**

I. Andonova, V. Ganev

16.00-16.15 h **BRIEFLY ABOUT LITHIUM**

D. Dimitrov

16.15 – 17.00 h – POSTER SESSIONS A AND B

Wednesday , November 25th

Session C: METAL TOXICITY AND CANCEROGENICITY. METALS AND ENVIRONMENT – PROBLEMS AND THEIR SOLUTIONS

Chairpersons:

Assoc. Prof. Anna Damianova, MD, PhD
Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences

Assoc. Prof Anastasia Daskalova, PhD
National Research Station for Wildlife Management, Biology and Pathology

Irena Andonova, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

10.00-10.30 h HEAVY METALS IN THE HOST-PARASITE SYSTEM OF HARES IN BULGARIA

M. Gabrashanska, V. Nanev, M. Anisimova, V. Ermakov, S. Tyutikov

10.30-10.45 h HEAVY METALS AND THEIR HARMFUL EFECTS ON THE LIVING CELLS

Y. Gluhcheva, M. Madzharova, E. Pavlova, B. Nikolov, N. Atanassova, M. Bakalska

10.45-11.00 h IMPACT OF COBALT ON MALE FERTILITY

M. Madzharova

11.00-12.30 h POSTER SESSIONS C AND D

12.30 -13.30 h LUNCH TIME

Session D: TRACE ELEMENTS AND MINERALS

Chairpersons:

Assoc. Prof. Margarita Gabrashanska, DVM, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

Assoc. Prof. Anna Tolekova, MD, PhD
Faculty of Medicine, Trakya University, Stara Zagora

13.30-14.00 h **EFFECTS OF SELENIUM SUPPLEMENTATION ON THE SERUM LIPID PEROXIDATION LEVEL AND ENOS-3 EXPRESSION IN AORTA AND MYOCARDIUM OF SPONTANEOUSLY HYPERTENSIVE RATS**

B. Ruseva, T. Betova, M. Alexandrova, A. Dimitrova, P. Laleva, V. Nikolov

14.00-14.15 h ***CLOSING REMARKS***

Session A. **Role of metals in normal and pathological processes**

Chairpersons:

Assoc. Prof. Yana Mizinska PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

Assoc. Prof. Reni Kalfin, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Assist Prof. Yordanka Gluhcheva, PhD
Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences

AO1. ABOUT IRON, PEOPLE AND LIFE

R. Alexandrova[1], R. Kalfin[2], O. Alexandrov[3], P. Borisova[4], E.-M. Mosoarca[5], R. Tudose[5], O. Costisor[5]

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Iron is essential for fundamental cell functions, such as DNA synthesis, transport of oxygen and electrons, and cell respiration. Iron deficiency is the most common known form of nutritional deficiency. Its prevalence is highest among young children and women of childbearing age. Iron deficiency states are associated with alterations in cellular function, growth, motor development, behavior and cognitive function. In pregnant women, it increases the risk for a preterm delivery and delivering a low-birthweight baby. On the other hand excess body iron can be highly dangerous because the metal is an effective catalyst in the free radical reactions. High tissue iron concentrations have been associated with the development and progression of several pathological conditions, including certain cancers, liver and heart disease, diabetes, hormonal abnormalities, and immune system dysfunctions.

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AO2. LET US REMEMBER ZINC

R. Alexandrova[1], M. Georgieva[1,2], T. Todorova[2], D. Culita[3], G. Marinescu[3], L. Patron[3]

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The importance of zinc was first documented for *Aspergillus niger* in 1869 [1]. It took over 75 years to realize that zink is also an essential trace element for rats [2] and an additional 30 years went before it was recognized that this was also true for humans [3,4]. Today it is well known that zinc is an essential ion in cells; without it cells cannot sustain life. Zink is a cofactor for more than 300 enzymes, representing more than 50 different enzyme classes, and is essential for cell growth. The elemnt is involved in protein, nucleic acid, carbohydrate, and lipid metabolism, as well as in the control of gene transcripotion, differentiation, development, and growth [5, 6]. The Recommended Daily Allowance (RDA) for zinc is 12-15 mg/kg, in balanced diets this amount is obtained by eating meat and other sources of animal products [7]. Zinc deficiency leads to a retardation of growth development in children, retarded development and hypoginadism, dermatitis and delayed wound healing, alopecia, poor pregnancy outcomes and teratogeny, and decreased immune function with a resulting increased susceptibility to infections [5, 8]. The prevalence of zinc deficiency is estimated to be high, with billions of people at risk, in particular in the developing world. In industrialized countries, elderly people are a high risk group for zinc deficiency [8, 9]. Many studies were published in which the effect of nutritional zinc supplementation on the incidence or severity of a certain disease was investigated [10].

Acknowledgements: Supported by Grant DOO-2-39/12.03.2009 between Bulgarian Ministry and Education and Science and Romanian Ministry of Education and Research

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AP1. ZINC FINGERS-ROLE IN RESTRICTION ENZYMES AND GENE THERAPY

T. Todorova, M. Georgieva

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Zinc, also known as spelter, is a metallic chemical element; it has the symbol Zn and atomic number 30. It is the first element in group 12 of the periodic table. One of the essential biological roles of zinc is its participation in DNA- binding domains called zinc fingers.

Zinc fingers are small protein domains that can coordinate one or more zinc ions to help stabilize their folds. They can be classified into several different structural families and typically function as interaction modules that bind DNA, RNA, proteins or small molecules.

Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target desired DNA sequences which enables zinc-finger nucleases to target unique sequence within a complex genome. By taking advantage of endogenous DNA repair machinery, these enzymes have become useful reagents for manipulating genomes of many higher organisms including *Drosophila melanogaster, Caenorhabditis elegans*, tobacco, rats, various types of mammalian cells, and zebrafish. An ongoing clinical trial is evaluating ZFNs that disrupt the CCR5 gene in CD4+ human T-cells as a potential treatment for HIV/AIDS. *Drosophila melanogaster*, *Caenorhabditis elegans*, tobacco

Zinc-finger nucleases (ZFNs) are powerful tools for experimental gene manipulation. A number of recent papers have shown how this technology can be applied effectively to models of human gene therapy. Significant target genes and useful methods of ZFN delivery have been reported. Important strides have been made in minimizing toxic side effects observed with some ZFNs, which bodes well for their ultimate safety. New tools are available for the design and testing of ZFNs for new target genes. Applications of ZFNs to stem cells have been described, and genuine gene therapy trials appear to be on the immediate horizon.

An efficient method for making directed DNA sequence modifications to plant genes (gene targeting) is at present lacking, thereby frustrating efforts to dissect plant gene function and engineer crop plants that better meet the world's burgeoning need for food, fibre and fuel. Zinc-finger nucleases (ZFNs)-enzymes engineered to create DNA double-strand breaks at specific loci-are potent stimulators of gene targeting; for example, they can be used to precisely modify engineered reporter genes in plants. Herbicide-resistance mutations were introduced into SuR(tobacco acetolactate synthase genes (ALS SuRA and SuRB)) loci by ZFN-mediated gene targeting at frequencies exceeding 2% of transformed cells for mutations as far as 1.3 kilobases from the ZFN cleavage site. More than 40% of recombinant plants had modifications in multiple SuR alleles. The observed high frequency of gene targeting indicates that it is now possible to efficiently make targeted sequence changes in endogenous plant genes.

References:

Guerts, A.M., et. al, Knockout Rats via Embryo Microinjection of Zinc-Finger Nucleases. Science. Vol 325, 24 July 2009 page 433 . (2009)

*D. Carroll (2008). "Progress and prospects: Zinc-finger nucleases as gene therapy agents". Gene Therapy **15**: 1463–8. doi:10.1038/gt.2008.145. http://www.nature.com/gt/journal/v15/n22/abs/gt2008145a.html.*

*S.C.Ekker (2008). "Zinc finger-based knockout punches for zebrafish genes". Zebrafish **5**: 1121–3. doi:10.1089/zeb.2008.9988. http://www.liebertonline.com/doi/abs/10.1089/zeb.2008.9988*

AP2. MATRIX METALLOPROTEINASES AS BIOMARKERS IN BREAST CANCER AIMING FACILITATING EARLY PROGNOSIS AND SUCCESSFUL TREATMENT

M. Georgieva

Faculty of Biology , Sofia University "St. Kliment Ohridski", 1164 Sofia, 8 Dragan Tsankov Blvd.

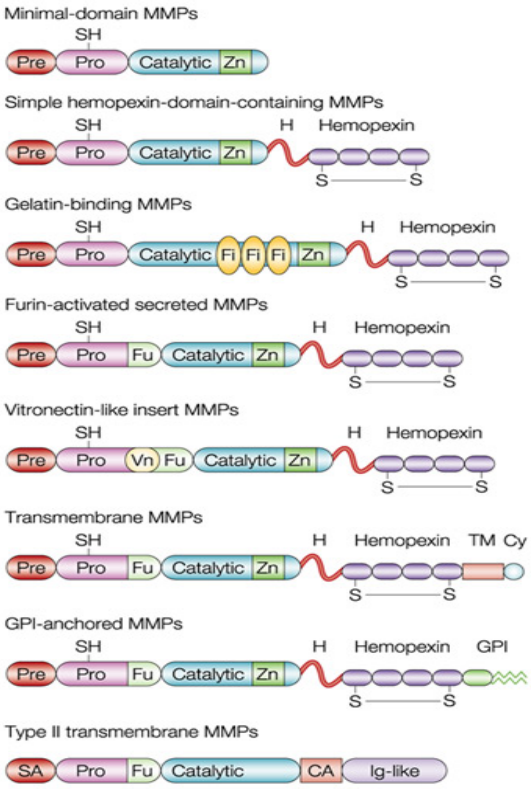
Breast cancer is the most common malignancy in females. Most of the lethal cases are cost by distant metastasis from primary tumor site, spreading to other areas of the body trough the bloodstream or lymphatic channels. To decrease mortality rates it is crucial to detect the tumor and metastasis at an early stage.

The metastatic process involves intravasation and extravasation of tumor cells, followed by reimplantation of tumor cells, formation of a new tumor stroma and neoangiogenesis. These steps in the promotion of malignant tumors require the involvement of proteolytic enzymes – matrix metalloproteinases, which can degrade the protein components of the extracellar matrix (ECM).

Recently, the level of active metalloproteinases (MMPs), especially MMP-2, is also considered to be a breast cancer metastasis indicator along with lymph node status, estrogen and progesterone receptor level, HER-2/neu gene amplification, overexpression of Bcl-2 or p53 etc.

Detection of active MMP-2 in circulating blood could be more sensitive than other, well-established methods. Measurements of the active MMP-2 level in this area could also provide important information necessary for beneficial treatment. Some authors describe patients with overexpressed MMP-2 as "high-risk group".

Human matrix metalloproteinases are a family of over 22 different endopeptidases that are able to degrade various components of the ECM. They play an important role in diverse physiological and pathological processes. The basic structure of these enzymes consists of three domains common to all of them:



- ***predomain*** – rapidly cleaved after cellular secretion as zymogens;
- ***prodomain*** – maintains enzymatic activity;
- ***Zinc atom containing domain*** – proteolytic release of the zinc atom leads to activation of the catalytic site.

Activation rate of pro-MMP-2 and active MMP-2 is used as an indicator of cancer metastasis. A high level of MMP-2 has been shown to predict adverse outcome in patients with gastric, pancreatic, and prostate cancers as well. MMP-9 (Gelatinase-B) / MMP-2 (Gelatinase-A) ratio was enhanced in cancer patients compared with those with benign diseases and healthy individuals.

To sum up, MMP-2 has reason to be an assisting marker in breast cancer metastasis diagnosis. Inhibition the MMP-2 activity or even synthesis by one or a combination of different agents that increase treatment efficacy may result in **metastasis suppression**.

References:

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2. Agnieszka Jezierska, Tomasz Motyl ***"Matrix Metalloproteinase-2 involvement in breast cancer progression: A mini-review"*** *Med Sci Monit, 2009; 15(2): RA32-40*

3. PA Arakaki, M R Marques and M C L G Santos ***"MMP-1 polymorphism and its relationship to pathological processes"*** *J.Biosci. 34(2), June 2009, 313-320*

4. Mikala Egeblad & Zena Werb, ***"New functions for the matrix metalloproteinases in cancer progression",*** *Nature Reviews Cancer 2, 161-174 (March 2002)*

АР3. МАГНЕЗИЙ И ЦИНК

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AP4. METALLOTHIONEIN AS MEDIATORS OF THE RESISTANCE IN ANTICANCER THERAPY

P. Mitrenga, G. Taleva

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Metallothioneins are ubiquitous group of cysteine-rich proteins with low molecular weight (3500 -14 000 Da) and capacity to bind heavy metals. They form complexes with physiological (such as zinc and copper) and xenobiotic (mercury, arsenic, cadmium) metals, via the thiol groups of the cysteine residues. The function of these proteins is not quite understood yet, but research data suggest they play an important role in the metabolism of physiological metals (Zn, Cu), and provide protection against metal toxicity and oxidative stress. There are four main forms in humans-MT1 MT2, MT3, MT 4.

Metallothioneins (MT) have been proposed to play an important role in carcinogenesis and anticancer therapy. A correlation between amplification of oncogenes and synthesis of some metallothioneines has been found. Studies have also reported increased expression of MT-I +II mRNA and proteins in various human cancers. The increased levels of MT are mechanism of resistance to anticancer drugs, as intracytoplasmic binding of MT prevents the active molecules from reaching their target, the intranuclear DNA of tumor cells. The thiol groups of these proteins are able to bind one of the most used cytotoxic agents, such as platinum compounds and alkylating agents. The interaction of MT with cytotoxic agents is not limited to covalent binding. Furthermore, the cytotoxic drugs are bound by MT after competition with zinc and copper. These metals are cofactors of numerous metalloenzymes. Competitive displacement of these metals could provide a zinc cofactor reserve that increases the cell's reparative potential when faced by DNA damage by cytotoxic agents.

Radiotherapy is also affected by MT. They act as scavengers of free radicals produced by irradiation. A number of *in vitro* and *in vivo* studies have linked overexpression of cellular MT with tumor cell resistance to radiation.

MT synthesis can be easly induced by physiologic heavy metals (such as zinc and copper). Pharmacological modulation of MT levels has been used to increase the MT pool in normal tissues and decrease their susceptibility to the toxicity of anticancer drugs. In the case of tumors arising in the brain, where the inducibility of MT synthesis is low, this approach would allow protection of normal tissues without decreasing the antitumor activity of the cytotoxic agents.

Evaluation of the interaction between MT and chemotherapeutic agents may be important in cancer treatment, where many questions about the role of MT as mediators of the resistance to the cytotoxic effects of anticancer drugs still remain unclear.

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AP5. SUPEROXIDE DISMUTASE AND CANCER

T. Jivkova[1], L. Dyakova[1,2]

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The superoxide dismutase (SOD) family of proteins is necessary to protect oxygen-utilizing cells from the toxicity of the reactive oxygen species (ROS) produced during normal metabolism. They catalyze the breakdown of superoxide into hydrogen peroxide and water and are therefore central regulators of ROS levels. Besides being protective proteins, these enzymes are also key components of signaling pathways that regulate cell physiology. There are three known forms of SOD in mammalian cells: a copperand zinc-containing superoxide dismutase (CuZnSOD) found mainly in the cytoplasm and nucleus, a manganese-containing superoxide dismutase (MnSOD) found in the mitochondria, and an extracellular superoxide dismutase (ECSOD) found primarily in the extracellular compartments. MnSOD is mitochondrial enzyme that disposes of superoxide generated by respiratory chain activity. Cancer cells are nearly always low in MnSOD and catalase (CAT) activity, and usually low in CuZnSOD activity. Recently, it has been shown that in some cancer cells, reduced expression of MnSOD is due to mutations in the promoter of the gene, while in other types of cancer, reduced levels of MnSOD are due to abnormal methylation, loss of heterozygosity, or mutation in the coding sequence. Thus, MnSOD loss has been suggested to be similar in mechanism to that reported for other tumor suppressor genes.

AO3. THE AFFECT OF COBALT SALTS ON SOME WEIGHT INDICES IN DEVELOPING MICE

Y. Gluhcheva[1], M. Madzharova[1], V. Atanasov[2], R. Nizamova[2], M. Mitewa[2], E. Pavlova[1]

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Although cobalt is as essential trace element long-term exposure and large amounts of its salts can have deleterious effects on humans and animals. Since it can be found in the environment, food and water exposure to this metal is unavoidable. Cobalt accumulates in

organs such as spleen, kidney, heart and liver. Its salts are shown to affect body weight of patients and experimental animals but the mechanism remains to be elucidated. Data show significant weight loss, as well as decreased food and water consumption in diabetic rats treated with cobalt chloride [2, 3]. The aim of the present study is to investigate the effect of organic and inorganic cobalt compounds on some somatic indices in developing mice – body weight, liver and spleen weight. Pregnant balb/c mice in late gestation were subjected to cobalt chloride ($CoCl_2.6H_2O$) or cobalt EDTA (Co-EDTA) treatment at daily doses of 75 mg/kg or 125 mg/kg. Cobalt salts were dissolved and obtained from drinking tap water. Sodium EDTA (Na-EDTA) and pure tap water were used as controls. Animals were fed a standard diet and had access to food *ad libitum*. Mice were maintained in individual standard cages to ensure that all experimental animals obtained the required dose of cobalt salts. The newborn pups were sacrificed on days 18, 25, 30, 45 and 60 which correspond to different stages of development. Mice were weighed weekly and the experimental cobalt concentration was adjusted accordingly. Preliminary results showed that mice treated with cobalt salts ($CoCl_2$ and Co-EDTA) have smaller body weight compared to the control group. Liver weight was increased in the Co-EDTA-treated mice for both doses and in all but d60 experimental groups. Spleen and liver weight was increased in case of high dose $CoCl_2$-treated mice. Spleen weight was the largest in high dose $CoCl_2$ –treated mice compared to all other groups. Liver weight of mice treated with Co-EDTA was the largest in all experimental groups compared to that induced by the other substances and in the control. The experimental results show that organic and inorganic cobalt salts affect body and organ weight. Data are in agreement with Garoui et al. [1] showing retarded weight gain in suckling rats. Further studies regarding cobalt bioaccumulation and its cytotoxicity in liver and spleen will be performed.

Acknowledgement: This work is supported by a grant No DO02-351/2008 for Young scientists from NSF.

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AO4. EFFECTS OF METALS ON BONE MINERALIZATION

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Bone formation requires calcium and phosphate. Vitamin D through its active metabolites provides for adequate amounts of calcium and phosphate in addition to regulating the differentiation and function of the bone cells involved. Osteomalacia results from the reduction in matrix mineralization, decreased mineral apposition rate and active mineralizing surface area. Metals that result in deficiencies in calcium, phosphate and active vitamin D metabolites or that interfere with their deposition in or action on bone could lead to osteomalacia.

Phosphate deficiency can be induced by ingestion of aluminum-containing antacids that prevent its absorption from the intestine or by proximal renal tubule toxins such as cadmium that result in renal phosphate wasting. Such patients present with little or no urine phosphate excretion and increased $1,25(OH)_2D$ levels. Serum and urine calcium levels tend to be high. Unlike these patients, patients with aluminum-induced osteomalacia generally have normal or even high serum phosphate levels and low $1,25(OH)_2D$ levels. The absence of aluminum in bone biopsy sample helps to identify those patients who develop osteomalacia on the basis of antacid-induced hypophosphatemia rather than aluminum-induced inhibition of mineralization. Heavy metals - lead and mercury cause accumulation of calcium in cells and disturb biochemical balance of bone.

Strontium is able to increase trabecular bone volume when administered at low concentrations. The metal increases bone mass by both inhibition of bone resorption and stimulation of bone formation. Treatment with strontium prevented bone loss, measured as bone ash and restore bone mineral content against the values in placebo-treated. Histomorphometric data and biochemical markers proved that strontium salt decrease resorption without reducing formation. Low doses of strontium or fluoride increase the number of bone-forming sites without detectable adverse effects on bone mineral content or bone matrix mineralization.

AP6. THE ROLE OF CALCIUM IN THE LIVING ORGANISMS

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In the present investigation the modern and actual problems about the organism - calcium interactions are discussed.

The aim of our study was to estimate and analyse the data of some authors concerning the role of calcium in the living organisms in our planet.

The investigations performed have suggested that calcium is a major essential element for living organisms in our planet. This soft grey alkaline earth metal was first isolated and named in 1808 by Sir Humphry Davy. Calcium is not naturally found in its elemental state. It is the fifth most abundant element by mass in the Earth's crust. Calcium occurs in sedimentary rocks in the minerals calcite, dolomite, gypsum; in igneous and metamorphic rocks chiefly in the silicate minerals: plagioclase, amphiboles, pyroxenes and garnets.

Calcium is an important mineral necessary for life and protection of health of living organisms in our planet. In plants Ca^{2+} ions are essential components of plant cell walls and cell membranes. They act in a number of signal transduction pathways. Some plants accumulate calcium in their tissues. In plants calcium is stored as Ca – oxalate crystals in plastids. Calcium is essential for vertebrates and invertebrates. Pearls are a gem produced by the metabolism of molluscs. They are composed 90 % of calcium carbonate and about 5 % of protein. The egg shell of birds is approximately 95% calcium carbonate crystals, which are stabilized by a protein matrix. Calcium plays an important role in physiology and biochemistry of organisms and cells and in signal transduction pathways. It regulates the transport of ions across cell membranes and plays pivotal role in nerve transmission, muscle contraction and clotting of blood. Calcium is essential to the formation of bones and teeth. It is the fifth most abundant element by the mass in human body. About 99 % of the calcium in the body is in the bones and teeth and 1 % is in the fluid that bathes the cells. In all tissues of

humans and animals it are found as phosphates and carbohydrates. Hydroxylapatite is the mineral portion of human and animal bones and teeth. Abnormal growth of these crystals in the body results in great number of diseases (pseudo-gout, urinary calculi, gall-stones, dental tartar, chondrocalcinosis, exostosis etc.). The important medical disorders are hypercalcemia and hypocalcemia. Long-term calcium deficiency can lead to rickets of csteoporosis. In Osteoporosis the bone mineral density is reduced and in the bone becomes porous and brittle.

This actual problem of calcium-organism interactions are related to modern biology, biomineralogy, medicine, pharmacology and protection of life in our planet.

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AP7. NICKEL AND ALLERGY

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Nickel allergy is still the most common contact allergy in Europe in spite of full implementation of the EU Nickel Directive in 2001. It is a chronic and recurring skin problem that may develop at any age. Once developed, nickel allergy tends to persist life-long. Epidemiologic studies have shown a sensitivity frequency of 20% in young females and 10% in the elderly. Two to four percent of males are sensitized. Nickel allergy is highest among females and patients under the age of 18, affecting 35.8% of patients patch-tested in this dermographic.

Nickel is a contact allergen causing Type 1 and Type IV hypersensitivity, mediated by regains and allergen-specific T lymphocytes. Most cases of nickel allergy can be related to skin contact with nickel containing metallic items as buttons, suspenders, ear ornaments etc. Nickel allergic contact dermatitis most commonly presents as a skin rash in areas exposed to nickel, however, more serious reactions to nickel in medical devices and more widespread eruptions to dietary nickel can occur. Some people who are sensitive to this element have asthma attacks following exposure to this element.

The biological significant parameter is not the nickel concentration in the alloy or coating but the amount released to the skin during exposure to human sweat. Nickel sensitivity may be avoided by restricting contact with objects that release nickel ions through sweat on skin.

Although nickel-based (Ni-Cr and Ni-Cr-Be) alloy prothesis is widely used in orthodontics, its potential biologic hazards, hypersensitivity in particular, are still uncertain as yet. There are data that exposure to nickel-containing orthodontic appliances may cause intra or extraoral allergic reactions incuding diffuse erythema, edema, eczema, fissuring, desquamation, and symptoms such as itching and soreness. Caution and close monitoring

should be exercised when placing nickel-containing orthodontic appliances in patients with known histories of nickel contact dermatitis.

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AP8. SPECTROPHOTOMETRIC AND CONDUCTOMETRIC STUDY OF THE COPPER(II) COMPLEXE OF A TRISAZOCOMPOUND DERIVED FROM 4,4'-DIAMINOBENZANILIDE

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The study of binding abilities of compounds containing enol-azo as functional groups is relevant because of their analytical use as chromogenic agents or extractants, as well as to their use as model for some metal-enzyme interactions and for the possible transport of metal ions in biological systems. Further, sulfonated disazo compounds have been studied as HIV inhibitors of viral replication due to their binding to both protease and reverse transcriptase of this virus. In the present work, the reaction of a trisazo sulfonated ligand derived from 4,4'-diaminobenzanilide and 1-amino-6-naphtol-3,6-disulfonic acid (L) with Cu(II) ion in aqueous solution was studied spectrophotometrically and conductometrically. For this purspose, the electronic absorption spectra of (L) in pure organic solvents of different polarities (such as: methanol, acetone, ethylene glycole, CCl_4 and DMSO) have been studied. Further, the pKa of L was determined from its spectra in buffer solutions (acetate, borate and phosphate) of varying pH (2÷7). The cu(II) complexe of (L) was also studied by means of UV-Vis spectroscopy. Its stoichiometry was determined using mole ratio, spectrophptometric methods and conductometric titration. The spectrophotometric methods indicated the formation of 1:1 and 1:2 (m:L) complexes. The analytical data indicated that the sulfonated trisazo compound (L) can be used as an indicator for the spectrophotometric determination of Cu(II) ion.

AP9. THE MISTERY OF PRION PROTEIN

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Prion related protein (PrPc) is a 32 kDa, Cu(2+)-binding glycophosphatidylinositol (GPI)-anchored sialoglycoprotein expressed on neurons, glia and a variety of non-neuronal tissue.

The mature PrP species consists of an unstructured N-terminal region of about 100 amino acids and a C-terminal segment, also around 100 amino acids in length. The N-terminus contains the five octapeptide-repeat region, which has a tight binding site for a single Cu2+ ion. A second tight copper site is present downstream of the octapeptide repeat region but before the structural C-domain. The remaining ~ 100 amino acids at the C-terminus are folded into a series of three α-helices and a small two-strand β-sheet and are stabilized by a disulphide bond linking residues Cys179 and Cys214.

Despite numerous efforts to elucidate its physiological role, the exact biological function remains unknown. Today it is known that PrPc participates in cell survival, differentiation and angiogenesis. PrPc also protects cells against oxidative stress and it seems to be involved in neuroprotection. Several studies have demonstrated that PrPc prevents cells from apoptosis and subsequent tissue damage. Moreover, PrPc plays an important role in the immune response.

When misfolded, is responsible for a number of transmissible spongiform encepthalopathies (TSEs). The key event in TSE aetiology is the conversion of normal, cellular PrPc to an alternate isoform PrP(Sc), characterized by increased beta-sheet content, resistance to proteases and detergent insolubility. Examples of the TSEs include Creutzfeldt-Jacob disease and kuru in humans, scrapie in sheep and goats, chronic wasting disease in deer and elk and bovine spongiform encephalopathy in cattle (mad cow).

Prion protein is not expressed only in mammals but also in other species such as birds, reptiles and fishes. However, it is noteworthy to point out that prion diseases are only observed in mammals while they seem to be spared to other species.

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Session B. **Metal compounds as potential therapeutic agents**

Chairpersons:

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Prof. Mariana Mitewa, PhD, DSc
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BO1. MICROBIOLOGICAL ACTIVITY OF THE POLYETHER IONOPHORE MONENSIC ACID AND ITS METAL COMPLEXES

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Polyether monocarboxylic antibiotics, known as monovalent ionophores, are extensively used in veterinary medicine for treatment of coccidiosis and diseases caused by some Gram-positive microorganisms. Ionophores as monensin, salinomycin, maduramycin readily bind monovalent metal ions as Na^+, K^+ etc, thus forming neutral metal complexes able to cross the cellular wall of bacteria. The antimicrobial mode of action of these antibiotics is due to disturbance of metal homeostasis in the intracellular space of bacteria cell. Consequently it causes an activation of variety of processes leading to ultimate cell death.

The antimicrobial properties of ligands, their structurally characterized complexes and the corresponding metal salts were studied against gram-positive bacteria using the double layer agar hole diffusion method. In order to have a deep insight into the reactivity of monovalent ionophores, we initiated a wide research on their complexation with divalent metal ions.

Results of our group have shown that the antibacterial activity of the polyether ionophore monensic acid could be enhanced via its coordination with some divalent metal ions [1-4]. In the present study the results on the interaction of monensic acid A (MonH) with Ca^{2+} and Mg^{2+} and on the structural characterization of the complexes obtained are reported (Fig. 1). The new metal complexes were tested to evaluate their bactericidal properties against Gram-positive microorganisms. The antimicrobial activity of both complexes is higher comparing to the non-coordinated MonH against *Bacillus subtillis*, *Bacillus mycoides and Sarcina lutea.*

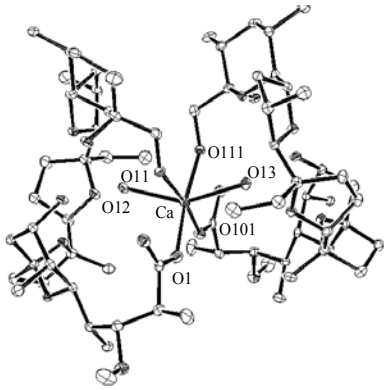

Fig. 1. ORTEP drawing of complex $[Ca(Mon)_2(H_2O)_2]$ at the 30 % probability level

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BP1. SILVER IN MEDICINE

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Silver is a metallic chemical element. The root of the chemical symbol even comes from the Roman word for silver: argentum (Ag). Pure silver has a brilliant white metallic luster. It has the highest electrical and thermal conductivity of all metals, and possesses the lowest contact resistance.

Silver has long been valued as a precious metal, and it is used to make ornaments, jewelry, coins. Today silver metal is also used in electrical contacts and conductors, in mirrors and in catalysis of chemical reactions. Its compounds are included in photographic film and dilute silver nitrate solutions and other silver compounds are used in disinfectants and microbiocides.

The evidence dates the knowledge of silver all the way to 5000 BC. Because silver has such a long history, there is no exact discover of silver, but rather a time period in history where evidence shows knowledge of silver. Silver has also a long and interesting history as an antimicrobial agent. Silver products have been used for thousand of years for their benefical effects, often for hygiene and in more recent years as antimicrobial on wounds from burn , trauma and diabetic ulcers. Early American pioneers moving West across the continent put

silver coins in large wooden water casks to provide safe drinking water for their long journey. Alexander the Great (356-323 BC) was said to drink only from silver vessels, and this is attributed (more recently) to the antimicrobial activities of the released Ag+ cations. The Romans used silver nitrate therapeutically and silver was entered in the official Roman book of medicines. The alchemical writings of Paracelsus (1493- 1541 A.D.) speak of the virtues of silver as a healin substance.

Silver is a broad spectrum agent: it is bactericidal to large number of Gram-positive and Gram-negative microorganism, many aerobes and anaerobes and several antibiotics-resistant stains. It has been developed for use in water purification, wound care, bone prostheses, reconstructive orthopedic surgery, cardiac devices, catheters and surgical appliances. This metal is often used as an alternative disinfectant in applications in which the use of traditional disinfectants such as chloride may result in the formation of toxic by-products or cause corrosion of surfaces. Silver has also been demonstrated to produce a synergistic affect in combination with several other disinfectants. Silver sulfadiazine creams (Silvazine and Flamazine) are topical ointments that are marketed globally. In recent years, a range of wound dressings with slow-release Ag components have been introduced. They are generally accepted as useful for control of bacterial infections as well as against fungi and viruses. NASA designed an ionizing copper-silver water sterilizing system for its Apolo flights. A Japan electric company used silver complex for inactivation of enveloped viruses.

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BP2. SILVER AND ITS USAGE IN WOUND TREATMENT

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Silver is a chemical element with the chemical symbol Ag (Latin: argentums) and atomic number 47. It has an essential role in biological systems. Silver is a broad- spectrum agent effective against a large number of Gram- positive and Gram- negative microorganisms, many aerobes and anaerobes, and several antibiotic- resistant strains such as methicillin-resistant *Staphylococcus aureus* and vancomycin- resistant enterococci.

Silver-containing dressings are widely used to assist with management of infected wounds and those at risk of infection. However, such dressings have varied responses in clinical use due to technological differences in the nature of their silver content and release and in properties of the dressings themselves. In a recently published study Silver examines the relationship between silver content, rate of silver release, and antibacterial activity in a simulated wound fluid model against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The study also looks at other important measures for the clinical performance of dressings including fluid-handling properties and dressing pH. Seven proprietary silver-containing dressings (AQUACEL® Ag [Hydrofiber®; "nonwoven A"], Acticoat™ Absorbent [alginate; "nonwoven B"], SILVERCEL™ [alginate-carboxymethylcellulose nylon blended fibers;

"nonwoven C"], Contreet® Foam [nonadhesive; "foam A"], PolyMem® Silver ["foam B"], Urgotul® S.Ag ["gauze"], and SilvaSorb® ["hydrogel"]) were assessed. No direct correlation between silver content, silver release, and antibacterial activity was observed. It was found that dressings with the highest silver content were nonwoven B and nonwoven C, while the lowest levels were found in nonwoven A and hydrogel. Nonwoven A, gauze, and nonwoven B were most effective against *S aureus* and *P aeruginosa*; however, their silver release rates differed widely. Free fluid absorption was greatest for the 2 foam dressings and least for gauze. However, nonwoven A and nonwoven B showed the best fluid retention under conditions of compression, while nonwoven A demonstrated the lowest level of capillary wicking. Dressing choice is a vital part of the successful management of infected wounds and those wounds at risk for infection. It has been suggested that dressing selection should be based on the overall properties of the dressing clinically relevant to the wound type and condition.

To date, no common wound pathogens have demonstrated resistance to pure silver. A Salmonella strain (not a wound pathogen) has shown resistance to pure silver. But many metal cations (Cd^{2+}, Hg^{2+}, Pb^{2+}, and Tl^{+} are examples in addition to Ag^{+}) are toxic and nonessential; and bacterial cells have genetically- determined resistance system to each. Bacterial silver resistance is frequently encoded by genes located on plasmids, but also sometimes found encoded on the chromosome. For example the silver resistance determinant studied in most detail was originally found on *Salmonella* plasmid pMG101. This determinant contains nine genes and the functions of eight named genes and their corresponding protein products have been assigned primarily on the basis of homologies to known proteins for other metal resistances. These genes on the plasmid are silE, silRS and silP, silCBA and silF. Genes on the chromosome are known as CusCBA and CusF. CusF and SilF are about 50% identical in sequence.

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BP3. CYTOTOXICITY AND EFFECTS OF 1,10-PHENANTHROLINE AND 5-AMINO-1,10-PHENANTHROLINE DERIVATIVES ON SOME IMMUNOCOMPETENT CELLS

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The biological activity of previously synthesized compounds [$(phen)_3(H^+)_2(NO_3^-)_2$ (**1**), Pd $(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ (**2**) and $Pd(phen)_2(NO_3)_2(H_2O)$ (**3**)] was investigated in vivo. The three compounds did not show any histological alterations in the observed lung, liver, spleen and lymph nodes of White Wistar rats. The propidium iodine staining did not discover any cytotoxic effect of the tested derivatives.

The tests for immunological response predominantly showed stimulation of the antibody-producing B-cells and lower or no stimulation of the T-cells. The LIF-test showed better stimulation of all lymphocytes with **1**, followed by **2** and **3**.
Substance 3 showed highest stimulating effect on B-cell blood lymphocytes in all doses (maximum in the lowest dose), whereas the impact of 2 is weaker and that of 1 is the weakest. The T-cell immune response after treatment with substance 1 is best influenced by dose of 1 mg in the spleen cell-fraction, followed by 3 (5 mg).

BO2. CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF RARE-EARTH METAL COMPLEXES WITH 4-HYDROXYCOUMARINS IN CELL CULTURES

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Coumarine derivatives have a broad spectrum of biological activities, including anti-HIV effect. Seventeen bis-(4-hydroxycoumarin) (4-hc) derivatives were synthesized in Bulgaria and later used as ligands. The complexes were characterized by different physicochemical methods: mass spectrometry, 1H NMR, 13C NMR, and IR spectroscopy. The spectra of the complexes were interpreted on the basis of comparison with the spectrum of the free ligands. First anti-HIV assays showing that three out of nineteen 4-hc had anti-HIV activity (IM-7, IM-8 and IM-10). This prompted us to prepare complexes with – cerium Ce(III), lanthanum – La(III) and neodymium – Nd(III) and to evaluate the cytotoxicity of the active derivatives on MT-4 cells and to study for anti-HIV activity in cell cultures. Cell toxicity (CC_{50}) and maximal non-toxic concentrations (MNCs) were performed in MT-4 cells on 72h by MTT-test. The MNC and CC_{50} for each compound were calculated from dose-response curves. The least cytotoxic compound according to MNC was IM-8, whose MNC values were higher than those of IM-7 and IM-10**.** Also, the least cytotoxic compound according to CC_{50} values was IM-8 for all derivatives. Detection of endogenous reverse transcriptase (RT) activity and RT processivity by PCR indicative for proviral DNA synthesis demonstrated that anti-HIV activity has not been linked to early stages of viral replication. The fact that no effect on RT and protease (expressing early and late stages of HIV-1 replication) was registered; one can suggest that RT and protease are not targets for the antiviral activity of IM-7-La. HIV-1 replication is too complicated and a number of targets and steps (except those studied here) should be taken into consideration (attachment, coreceptor binding, fusion, RNase H-activity, integration, inhibition of glycosylation or sialylation, etc). Although the complexes are of low potency against HIV-1 and should not enter clinical trials, the experience with them shows once more that the cytotoxicity could be reduced and the antiviral effect—highly expressed through complexation reactions.

BP4. COPPER AND ANTIANGIOGENIC ANTICANCER THERAPY

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Copper from cuprum, Cu is the 29th element of the Periodic Table with electronic configuration $3d^{10}4s^{1}$. Following zing and iron, it is the third most abundant trace element in the body. Critical proteins such as cytochrome oxidase, zinc–copper superoxide dismutase, lysyl oxidase and several transcription factors require copper for activity. Copper is involved in variety of biological processes such as embryo development, connective tissue formation, temperature control and nevre cell function.

Copper is normally absorbed by the intestinal tract and then enters the blood circulation through the action of ATP7A protein. Then it binds to human serum albumin and L-histidin that form an exchangeable pool of copper. The metal is transported via the portal blood to the liver. A large part of the copper in serum is bound to the ceruloplasmin, a multi-Cu oxidase, and its drop in the level is the first evidence of copper deficiency.

Angiogenesis, the formation of new capillaries from existing vasculature, is a critical process in normal physiology as well as several physiopathologies. The activators and inhibitors of angiogenesis can be divided into seven categories: growth factors, proteases, trace elements, oncogenes, cytokines, signal transduction molecules, an endogenous inducers. Depletion of copper has been shown to inhibit angiogenesis in a wide variety of cancer. So copper is a primitive growth factor. Copper stimulation of factors involved in vessel formation and maturation, such as vascular endothelial growth factor (VEGF), is mainly responsible for its angiogenesis effect. It leads to tumor growth, which allows the vessel to expand. Most of angiogenic promoters (VEGF) appear to be available for recruitment by various types of tumors and thus host cells assisting in the tumor growth and invasion process. Copper is required for the activation of hypoxia-inducible factor-1 (HIF-1), a major transcription factor regulating the expression of VEGF. Copper would be transported into nucleus by a copper chaperon for superoxide dismutase-1. Copper is required for HIF-1 interaction with the hypoxia-responsive element of the target genes and ensures the formation of HIF-1 transcriptional complex, thus activating the expression of target genes including VEGF. The essential role of copper in production of VEGF makes it implicated in anti-angiogenesis therapy.

Anticancer strategy rely on copper reduction, which inhibits the angiogenic activity. Wilson`s disease is an autosomal recessive disorder resulting in extreme accumulation of copper in the liver with deposits elsewhere in the body. Menkes is characterized by a systemic copper deficiency and is the result of an X-linked recessive mutation in a copper transporter. Studies have shown greatly elevated levels of copper in cancer tissues, and some diagnostics and treatments from Wilsons and Menkes diseases, such as copper chelation therapy, have been used in the treatment of cancer. There are three types of anti-cooper drugs: **penicillamine**, chelator - sulfhydryl group binds copper; clears copper through urinary excretion, used in liver disease; **trientine**, chelator - thiol groups tightly bind copper, inhibit angiogenesis and enchance apoptosis in tumor, used in pecicillamine intolerance and **tetrathiomolybdate**, which forms complex with Cu and protein, used in neurological involvement. The use of copper lowering as an anti-angiogenic strategy in the cancer chemopreventative setting remains to be investigated.

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BO3. COPPER CHELATION THERAPY AND CANCER TREATMENT

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The term "chelate, from the Greek "chele" for "claw", refers to the ability of a substance to combine with metalsin the body so that they can be excreted. Metal chelation therapy is traditionally used in occupational medicine, as it effectively removes toxic heavy metals from the body. Chelation therapy is also used as a complementary or alternative treatment in an attempts to inhibit oxidative stress, atherosclerosis or tumor growth. Metal chelation is also used to study the biological function of metal ions in living systems.

Copper is a tightly regulated trace element. Disruption of copper homeostasis are rare and they cause serious disorders such as Wilson's disease and Menkes diseas. Copper also plays an important role in promoting physiological and malignant angiogenesis. Formation of new blood vessles by a tumor enables cancer growth, invasion and metastasis.

Tetrathiomolybdate (TTM) is an anticopper drug initially developed to treat Wilson's disease, a genetic disorder characterized by excessive accumulation of copper in the liver and consequent liver and brain damage. TTM quickly and efficiently depletes copper stores and is under investigation as an anti-angiogenic agent. Promissing results in vitro, in pre-clinical animal models and in early (Phase I) clinical trial have led to the phase II evaluation of TM in patients with advancerd cancers.

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BO4. MELOXICAM AND METAL COMPLEXES OF MELOXICAM AS POTENTIAL ANTITUMOR AGENTS

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Cyclooxigenase (COX) inhibitors such as non-steroidal anti-inflammatory drugs (NSAIDs), already widely used to reduce fever, inflammation and pain, are under increasing consideration as potential agents for the prevention and treatment of neoplasia. Many reports have demonstrated that some NSAIDs suppress malignant transformation and tumor growth and are expected to be new anticancer agents. The promising results from pre-clinical studies have been reported for the selective COX-2 inhibitor meloxicam (Fig.1). The antineoplastic activity of this drug has been found to be in conjunction with reduced angiogenesis and induction of apoptosis. A potential clinical application of meloxicam in combination with cytotoxic drugs has been suggested. The radiosensitizing potential of of this drug has also been reported.

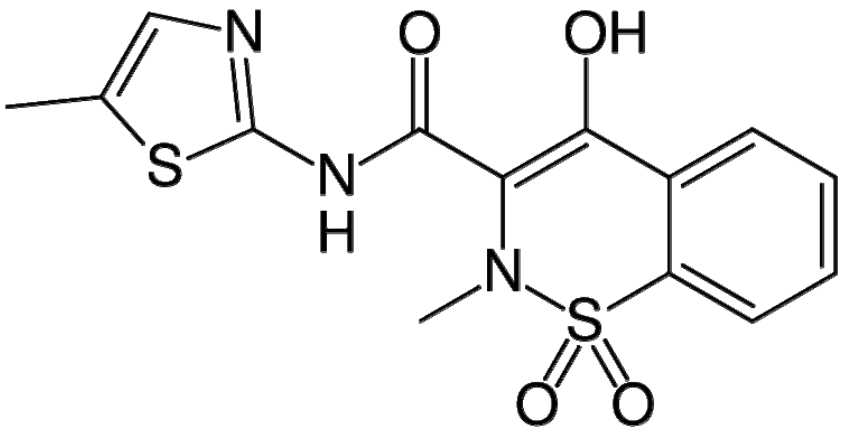

Fig.1 Meloxicam

The results from phase II study reported by Suzuki et al. (2009) demonstrate that meloxicam in combination with carboplatin and weekly paclitaxel chemotherapy was well tolerated and showed promising activity with encouraging survival in patients with advanced non small cell lung cancer (NSCLC).

It has long been suggested that the mode of action of many anti-inflammatory drugs may involve the chelation with some bioactive metals such as Cu(II) and Zn(II).

The aim of the study presented here was to evaluate the effect of metal complexes with meloxicam on viability and proliferation of cultured human tumor cells. The permanent cell lines 8 MGBA (glioblastoma multiforme), HeLa (cervical carcinoma) and HepG2 (hepatoma) were used as model systems in the experiments. The investigations were performed by MTT assay. The compounds were applied in concentrations of 1 – 200 μg/ml for 24, 48 and 72h. The results obtained revealed that the Cu(II) complex of meloxicam is more pronounced cytotoxic and antiproliferative agent as compared to meloxicam.

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BP5. IN VIVO STUDY ON ANTIOXIDANT PROPERTIES OF A NEW POTENTIAL ANTITUMOR AGENT SLCNUgly

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Backgrou nd. By *in vitro* investigations a good antioxidant activity was found for spin labeled amino acid 2-chloroethylnitrosourea, derivative of glycine (SLCNUgly), whereas such activity was no found for its clinically used analogous antitumor drug 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU).
Aim. To investigate in vivo antioxidant properties of SLCNUgly, formerly synthesized by us in relation with the presence of nitroxide moiety in its structure and to compare to those of the CCNU.
Materials and methods. The effect of SLCNUgly on the activities of antioxidant enzymes superoxide dissmutase (SOD) and catalase (CAT) and the level of lipid peroxidation (LPO) products in liver and kidney tissue, isolated from mice (n=18), treated by it was evaluated by spectrophotometric measurements at 3rd, 6th and 24th h after treatment. The same oxidative stress biomarkers have been also studied in mice (n=18), treated by antitumor drug CCNU and compared to those of mice treated by SLCNUgly. Control group (n=6) was inoculated with solvents only.
Results. The results are shown in table 1.

		CAT [kU/g Pr]			MDA[nM/g Pr]		
Controls		3rd h	6th h	24th h	3rd h	6th h	24th h
	Liver	12.2±0.6			1.07±0.24		
	Kidney	37.4±3.75			1.05±0.07		
SLCNUgly	Liver	15.9±2.0	16.2±1.9	11.8±1.6	0.51±0.04	0.54±0.1	0.68±0.14
	Kidney	11.2±1.04	16.3±3.7	12.9±3.0	1.64±0.5	1.38±0.2	1.06±0.2
CCNU	Liver	15.1±1.3	22.4±1.7	20.3±1.8	1.34±0.24	1.24±0.25	1.36±0.34
	Kidney	28.9±1.8	14.1±3.3	7.1±3.1	1.8±0.3	1.19±0.1	1.32±0.1

Discussion. CCNU increased lipid peroxidation in liver homogenates and the renal tissues. This is accompanied by activation of catalase in the liver, while renal activity of enzyme significantly reduced. Obviously, the effect on the kidneys due to the effects of metabolic products of the drug. It should be mentioned that at the end of the followed period LPO process persisted in the liver and kidney homogenates of mice treated by CCNU, whereas that process was attenuated in mice treated by SLCNUgly.

Based on the present results we assumed:

- Good antioxidant activity of SLCNUgly was most likely involved in scavenging and neutralizing of toxic free radical species, generated during its metabolism *in vivo*.
- The catalase activity of kidney was very sensitive to various toxic effects. Its inhibition contributes to maintaining a high lipid peroxidation in renal tissue.

BP6. WATER-SOLUBLE PALLADIUM(II) AND PLATINUM(II) COMPLEXES OF GLYOXYLIC ACID OXIME. STRUCTURE, MULTI-NMR SPECTRA, CYTOTOXIC AND APOPTOGENIC EFFECT

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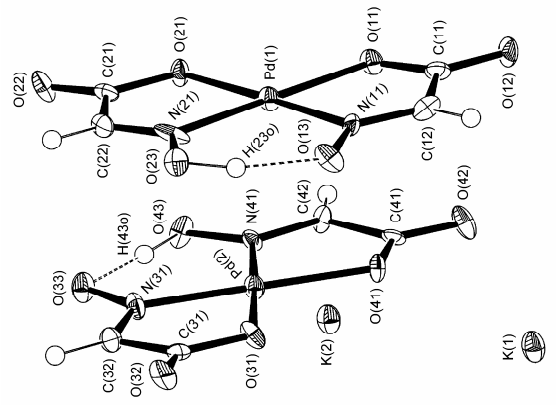
Several years ago we firstly described[1] Pd(II) and Pt(II) complexes of glyoxylic acid oxime (H_2GAO), and based on the detailed analysis of their vibrational spectra, suggested for them a bis-chelate structure with monodeprotonated ligands: [$M(HGAO_2$]. Later, K. Mereiter[2] solved the structure of the Pt(II) complex by X-ray crystallography, and found that it is actually an anionic complex, containing both mono- and di-deprotonated chelate ligands: $K[Pt(HGAO)(GAO)]\cdot 3/4H_2O$. The complexes mentioned bear some resemblance (*cis*-oriented nitrogen and carboxylato ligands) to the second generation platinum anticancer drugs carboplatin, nedaplatin and oxaliplatin[3], and hence, they are of interest as potential cytostatic agents. Here we report the X-ray crystallographic structure of the complex K[Pd(HGAO)(GAO)], multi-NMR spectroscopic data and results from cytotoxic activity assays of H_2GAO and its complexes K[Pd(HGAO)(GAO)] (**1**) and $K[Pt(HGAO)(GAO)]\cdot 3/4H_2O$ (**2**).

The orthorhombic crystals of **1** (*Pbca*, a = 15.890(2), b = 12.522(4), c = 16.703(3) Å, Z = 8) consist of two non-equivalent anionic complex molecules. Each complex molecule contains one mono- and one di-deprotonated H_2GAO molecules coordinated to Pd(II) *via* the carboxylato oxygen and oxime nitrogen atoms, forming two *cis*-oriented five-membered planar chelate rings. The two ligand molecules are connected *via* an intramolecular hydrogen bond of the type N−O···H−O−N (the Figure). The structure



obtained is very similar to that of the analogous complex **2**, deposited earlier. Complexes **1** and **2** were characterized by ^{1}H, ^{13}C and ^{195}Pt NMR spectra in water solution. Complex **2** exhibits a moderate cytotoxic activity (IC_{50} = 62 ± 16 μmol/l) and apoptogenic effect against human leukemic cell line K562. In comparison with cisplatin, the complex shows a lower level of necrosis on the same cells, and has higher aqueous solubility.

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BP7. NEWLY SYNTHESIZED POLYNUCLEAR ZN(II) COMPLEXES: INFLUENCE ON VIABILITY AND PROLIFERATION OF CULTURED TUMOR AND NON TUMOR CELLS

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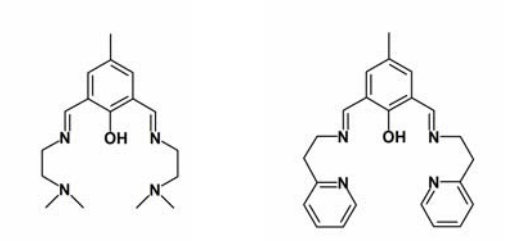
The Schiff base ligands provides high efficiency in building supramolecular architectures such as coordination polymers, helicate structures, grids, racks, clusters etc.[1] due to their ability to accommodate different metallic ions in their coordination sites. The ligands and the metallic ions, through their properties, can bring certain characteristics to the newly obtained self-assembling structure (molecular magnetism, photoluminescence etc.) and potential applications in therapy (antibacterial, antiviral, antifungal and citostatic agents),[2] homogeneous and heterogeneous catalysis.[3]

Here we report some biologic data of the newly polynuclear complexes of zinc(II), which are obtained employing binuclear complexes with Robson-type ligands as tectons. The self-assembly processes between binuclear $[Zn_2L^n]$ ($[Zn_2L^1(\mu\text{-}OH)(H_2O)_2](ClO_4)_2$ and $[Zn_2L^2(\mu\text{-}OH)(H_2O)_2](ClO_4)_2$) complex cations and *exo*-bidentate ligands [4,4'-bipyridine (4,4'-bipy), 1,2-bis(4-pyridyl)ethane (bpeta)] generate 1-D coordination polymers:

$_1^\infty$[{L^1Zn_2(μ-OH)}(μ-4,4'-bipy)]$(ClO_4)_2 \cdot 2H_2O$ **1**, $_1^\infty$[{L^1Zn_2(μ-OH)}(μ-bpeta)]$(ClO_4)_2$ **2**; $_1^\infty$[{L^2Zn_2(μ-OH)}(μ-4,4'-bipy)$_2$]$(ClO_4)_2 \cdot 2H_2O$ **3** (HL^n are compartmental Schiff-base ligands resulting from condensation reactions between 2,6-diformyl-*p*-cresol with, respectively, N,N-dimethyl-ethylenediamine and 2-aminoethyl-pyridine – Scheme 1).

Scheme 1.



HL^1 **HL^2**

The effect of the compounds on viability and proliferation of cultured tumor (A542, 8 MG BA, MCF-7, HeLa) and non tumor (MDBK, BALB/c 3T3) cell lines was evaluated by MTT test. The complexes were applied at concentrations of 1 – 200 μg/ml for 24, 48 and 72h.

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BP8. ADDITION COMPOUNDS OF BIS(SUBSTITUTED-SALICYLALDEHYDO) COBALT (II) WITH 2,2'-BIPYRIDINE: EFFECT ON VIABILITY AND PROLIFERATION OF CULTURED TUMOR CELLS

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The strong coordinating properties of 2-hydroxy-benzaldehyde (salicylaldehyde, Hsalo) and its derivatives with transition metals have stimulated research in these compounds, that find applications in both pure [1,2] and applied chemistry fields [3,4].

The cobalt(II) addition compounds [Co(X-salo)$_2$(bipy)], where X-salo is the anion of substituted salicylaldehydes (X = 3-OCH_3 (1); 5-Cl (2); 5-NO_2 (3)) and bipy the neutral 2,2'-bipyridine, were synthesized and characterized by physicochemical and spectral (IR, UV-Vis) data [5].

The aim of the present study was to investigate the putative cytotoxic and antiproliferative properties of these compounds on cultured human tumor cells. The following cell lines were used as model systems in the experiments:

- 8 MG BA (glioblastoma multiforme)
- MCF-7 (breast cancer)
- HeLa (carcinoma of the cervix)

The complexes were applied at concentrations of 1-50 μg/ml for 24h and 48h.
Cell viability was determined by MTT test. The compounds examined were found to decrease in a time- and concentration- dependent manner the viability and proliferation of the treated cells.
Acknowledgement: Supported by a bilateral project between Bulgarian Academy of Sciences and Aristotle University of Thessaloniki, Greece.

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BP9. CO(II) ADDITION COMPOUND WITH SALICYLALDEHYDE AND THE NITROGENOUS BASE PHENANTHROLINE: EFFECT ON VIABILITY AND PROLIFERATION OF CULTURED TUMOR CELLS

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Cobalt chelates with bidentate heterocyclic nitrogenous bases, such as 1,10-phenanthroline have been reported to possess important biological properties [1].

The cobalt(II) addition compound [Co(X-salo)$_2$(Y)], where X-salo is the anion of substituted salicylaldehyde (X = 5-NO_2 and Y= the neutral 1,10 phenanthroline (phen), was synthesized and characterized by physicochemical and spectral (IR, UV-Vis) data [2].

The aim of the study presented here was to evaluate the influence of this Co(n) complex on viability and proliferation of cultured tumor (8 MGBA, HeLa, MCF-7) and nontumor cells.

The investigations were performed using MTT assay. The results obtained revealed that both [Co(5-NO_2-salo)$_2$(phen)] and phenanthroline expressed significant cytotoxic and antiproliferative properties when applied at concentrations of 1-50 μg/ml for 24 h and 48 h.

Acknowledgement: Supported by a bilateral project between Bulgarian Academy of Sciences and Aristotle University of Thessaloniki, Greece.

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BP10. VIRUS-TRANSFORMED ANIMAL TUMOR CELLS: SUCCESSFUL APPLICATION FOR THE SCREENING OF NEW ANTINEOPLASTIC AGENTS

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It is widely accepted that tumor cell lines have played an important part in our understanding of cancer and have been used extensively in the discovery and characterization of new chemotherapeutic drugs. Two permanent cell lines established from transplantable tumors in chicken (hepatoma induced by the myelocytomatosis virus Mc29 – LSCC-SF-Mc29) and rat (sarcoma induced by Rous sarcoma virus strain Schmidt-Ruppin – LSR-SF-SR) have been applied in our investigations to evaluate the putative cytotoxic and antiproliferative properties of 54 newly synthesized compounds: transition metal complexes with various ligands (aminoacids, Mannich bases, cholic acids, mixed ligands) and metallacarboranes. The investigations were performed using MTT test, neutral red uptake cytotoxicity assay and colony-forming method. The results obtained revealed that LSCC-SF(Mc29) and LSR-SF-SR tumor cells could be considered as suitable test-systems for the initial screening of new potential antineoplastic agents because of the following main reasons: i) as compared to the other cell lines included in the experiments (obtained from human malignant neoplasms of the brain, breast, liver, larynx, cervix, lung) LSR-SF-SR and

especially LSCC-SF-Mc29 were found to be the most sensitive to the cytotoxic and antiproliferative properties of the compounds investigated; ii) primary cultures from various tissues of healthy chickens or rats could be easily prepared to serve as "controls" for comparative investigations with chicken hepatoma and rat sarcoma cells, respectively; iii) according to the literature available up to now the effect of synthetic substances and natural products on viability and proliferation of virus-transformed cells is not fully clarified; iv) the viral oncogenes v-myc (LSCC-SF-Mc29) and v-src (LSR-SF-SR) are integrated in genome DNA of chicken hepatoma or rat sarcoma cells, respectively. The cellular analogues of these oncogenes are proved to be involved in pathogenesis of a wide variety of human malignant neoplasms.

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BP11. EFFECT OF METAL COMPLEXES WITH MANNICH TYPE LIGANDS ON VIABILITY AND PROLIFERATION OF CULTURED GLIOBLASTOMA CELLS

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The incidence of primary brain tumors worldwide is approximately seven per 100 000 individuals per year, accounting for ~ 2% of primary tumors and 7% of the years of life lost from cancer before the age of 70. Brain tumors are the third leadingf cause of death from cancer in persons aged 20 to 39. Gliomas are the most common central nervous system (CNS) primary neoplasms in adults, consisting of 63% of all primary CNS tumors. They are classified into four clinical grades, grade 4 or glioblastoma multiforme (GBM) is the most aggressive of these tumors. The majority of these neoplasms remain difficult to treat because of the infiltrative growth of the tumor cells, and their resistance to standard therapy. Survival of patients affected by GBM has reminded virtually unchanged during the last decades (i.e. 6-12 months post-diagnosis) despite advances in surgery, radiation, and chemotherapy [1, 2].
Thirty compounds of Cu(I,II), Co(II), Ni(II) and Fe(II,III) with Mannich bases type ligands - N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN), were studied for their influence on viability and proliferation of cultured 8 MG BA human glioblastoma multiforme cells. The experiments were performed

using MTT test, neutral red uptake cytotoxicity assay, trypan blue dye exclusion technique, cvolony forming method and acridine orange staining. The results obtained revealed that: 1) Tested independently the ligands BAMP and TAMEN did not decrease significantly the viability and proliferation of 8 MG BA cells; 2) Among the compounds investigated the mixed ligand Cu(I,II) complex Cu_2BAMPdipyCl_4 (dipy = 2,2-dipyridyl) exhibited the most pronounced cytotoxic and antiproliferative properties; 3) All metal complexes examined were demonstrated to be less active as compared to cisplatin - the most widely used in clinical oncology anticancer agent.

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BP12. GOLD IN MEDICINE

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Man knows gold as one of the oldest precious metals but in his long and complex history it also plays a crucial pole in human medicine. There are a lot of myths and legends about this valuable metal in the ancient history. They present it not only as a sign of wealth among ordinary people but also as the glory of the immortals. It is highly priced because of its physical properties as softness, high density, resisting of corrosion and inertness.

The beginning of the gold medical history is Robert Koch's discovery of its effect in therapy for tuberculosis. He introduced that gold cyanide is considerably effective against *M. tuberculosis* in cultures. But it wasn't accepted as a proper treatment of pulmonary tuberculosis until 1925. Some other scientist like Holger Mollgaard, Secher and Faber also approved the effect of gold compounds and its use in clinical practice. Controversially other Danish physicians were concerned about their use in medicine because of the high toxicity they show. As a result of many additional clinical tests made in United States the treatment of tuberculosis with gold compounds was ceased because of its violent reactions. After that the interest of gold in medical use diminished until the 20th century. At this time some metal compounds was discovered to be eligible for curing rheumatoid arthritis. This disease is characterized by a movement of active phagocytes and other leukocytes into synovial and periarticular tissue. Rheumatoid condition is exacerbated and continued by the appearance of active oxygen species and other mediating substances from triggered phagocytes. As one of the metal compounds gold salts induced by lysosomes inhibit the phagocytes and by this reduce the toxic oxygen production. Gold was also widely used in dentistry for dental protheses because this metal has very low rate of corrosion, long half-life and sufficient strength. However nowadays gold protheses are less preferred because of esthetic concerns and cost. What is most interesting about gold compounds is their cytotoxicity and anti-tumor activity which was recently discovered. In 1980's some of the gold (I) complexes with phospine and thiolate ligands which were known as antiarthritic compound were also considered as anticancer agents. Several studies were performed with gold (I) complexes with

phosphin and thiolate ligands revealed that the cytotoxity is contributed by the phosphin ligand. The mechanism of their action is thought to be an interaction with DNA. The further investigations of their action indicate that gold complexes bind to DNA and cause conformational changes. With the emerge of the second- generation anticancer gold (I) complexes, e.g. tetrahedral four-coordinate gold (I) phosphine complex with chelated diphosphine ligands, was clarified the primary cellurar target to be mitochondria not DNA. The mechanism is not completely identified but is thought to cause an uncoupling of oxidative phosphorilation, release of ATP and cell death. Other mechanisms are also possible as producing highly reactive oxygen species, disabling the calcium regulation, increasing the permeability of mitochondria and inducing gene mutation. Several other attempts were made with gold (III) complexes as anticancer agents. They show high cytotoxic potency despite there were difficulties in synthesizing them. Some of the complexes that were tested *in vitro* are gold (III)polyamine complexes, gold(III) terpiridine, gold(III) phenanthroline and gold (III) glycylhistidine. Both gold (III) terpiridine and gold (III) phenanthroline lose their stability in the presence of proteins which makes them unsuitable for medical use. The mechanism of action of gold (III) complexes is considered as a simple binding to DNA. However these interactions between the complex and the DNA were revealed to be too weak to cause such a biological effect. All these discoveries of the potency of the gold prove that it is worthy to be further studied as an anticancer agent. They arise interesting questions about the mechanism of action of gpld complexes and inspire new ideas for developing a successful antitumor treatment .

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BP13. NANOPARTICLES AS DRUG DELIVERY AGENTS

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Nanoparticles are structures and devices with length scale in the 1-100 nm range. These objects take on novel properties and functions that differ from those seen in the bulk scale. Thanks to our ability to manipulate the physical, chemical and biological properties of the nanoparticles, we can design them for image contrast agents, diagnostic and drug delivery purposes.

Typical nanoparticle consists of a “core” (solid or liquid) and “corona” above the core. The corona can be functionalized with hydrophilic polymers (PEG, dextran, etc.), targeting molecules, therapeutic drugs, image contrast agents. Hydrophilic polymers increase the solubility and compatibility of the particle and act as a platform for lipophilic molecules.

Nanoparticle is a good vehicle for drug delivery because of their small size. They must be small enough to avoid rapid filtration by the spleen and avoid the Kupffer cells. Nanoparticles can penetrate through the blood-brain barrier, stomach epithelium and, when smaller than 20 nm, they can transit through blood vessel walls. In comparison with microparticles, nanoparticles don’t tend to stay where they are placed. On the contrary, they are rapidly cleaned by the

reticuloendothelial system. But when the nanoparticle surface is modified with hydrophilic agent, the circulation time of the particle can be greatly increased.

Nanoparticles are small enough to be pinocytosed by all cell types. Microparticles are phagocytosed only by antigene presenting cells, providing passive targeting to the macrophages, for example. Passive targeting is also when the particle is larger than 100-150 nm and tend to accumulate in tumor cells, because of the relatively "leaky" beds, but will not leave the normal blood vessels.

Active targeted delivery of nanoparticles can be accomplished by attaching a mAb or cell-surface receptors ligand that binds specifically to molecules found on the surfaces of targeted cells, mainly cancer cells. Targeting molecules that have been used successfully include folate, luteinizing hormone-releasing hormone (LH-RH), thiamine, receptor-specific peptides, aptamers and a wide variety of mAb directed against cell-surface markers, such as integrins. Once bound to the target cell, the nanoparticles are readily internalized by receptor-mediated endocytosis.

For intracellular drug delivery for DNA (gene delivery) and protein, and treatment of intracellular infections, the compound must be provided to specific sites within the cell, which sometimes is not an easy task. It also must be ensured that the payload is released before transcytosis/exocytosis expels the particle from the cell. For this purpose environment-sensitive materials are used. For example, the use of pH triggerable materials in lipid-polymethacrylate microspheres and nanoparticular liposomes.

Nanoparticles as drug delivery agents have become important in experimental pharmaceutics and medicine with their significant properties. Nanotechnology is relatively young but fast developing field with broad opportunities for future innovation.

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BP14. THE ROLE OF GOLD NANOPARTICLES IN THE DEVELOPMENT OF APPROVED CLINICAL DIAGNOSTIC METHODS

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Nanodiagnostics can be defined as the use of nano-sized materials, devices or systems for diagnostic properties. Gold nanoparticles (AuNPs) generally consist of either a thin gold shell surrounding a dielectric core (an insulating material, e.g. silica), referred to as nanoshells or just AuNPs (typically spherical in shape). They range in size from 0.8 to 250 nm and are characterized by high absorption coefficients. The size and shape of AuNPs determine their optical properties. AuNPs can be used in labeling DNA and proteins for detection of biological targets with enhanced sensitivity. They are primarily utilized in imaging, immunasaay, and molecular diagnostic applications. AuNPs are key components of the bio-barcode assay (BCA), which has been proposed as a future alternative to PCR, with zepromolar sensitivity for DNA detection. The BCA has also been used for protein detection with attomolar sensitivity. AuNPs are non-cytotoxic and have been suggested to be of utility for imaging applications.

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BP15. SUPERPARAMAGNETIC NANOPARTICLES

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Superparamagnetic nanoparticles are made of magnetic materials such as iron, nickel, cobalt, or alloys of magnetic metals. Superparamagnetic nanoparticles can be used in different fields such as:

1) Multiplex immunoassays;
2) As contrast agents in magnetic resonance imaging (MRI) where they have much quarter magnetic susceptibility and more widespread tissue distribution (e.g. iron oxide particles) than conventional MRI contrast agents such as gadolinium;
3) To separate pathogenic cells from normal cells. The pathogenic cells are labeled with superparamagnetic nanoparticles that are coated with antibodies specific to markers on these cells.

Acknowledgement: Supported by a bilateral project between Bulgarian Academy of Sciences and Romanian Academy

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Azzazy HME, MMH Mansour. Clinica Chimica Acta, 403, 2008, 1-8.

BP16. POTENTIAL THERAPEUTIC PROPERTIES OF PLASMONICALLY HEATED GOLD NANOPARTICLES IN HUMAN CELL LINES

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Introduction: Local application of heat is a well-known concept in therapeutic medicine that has been explored extensively for the treatment of cancer and other conditions. Energizing sources, such as infrared lamps, ultrasound or lasers, can be used in the process, but there is always the problem of limiting the heat generated to the region of the target tissue.

The plasmon resonance for ordinary gold nanospheres is at ~520 nm (corresponding to green light), in the middle of the visible spectrum. Nanoparticles of gold, which are in the size range 10–100 nm, undergo a plasmon resonance with light. This is a process whereby the electrons of the gold resonate in response to incoming radiation causing them to both absorb and scatter light.

The aim of the present investigation was to elucidate effect of different size gold nanoparticles (40 and 100 nm) alone, combined with laser irradiation or laser treatment only on permanent human cancer cell lines HeLa and Hep-2.

Material and methods: The gold nanoparticles (GNPs), used for treatment were 40 and 100 nm in diameter. The samples were irradiated with Nd:YAG laser system operated at λ=532nm and pulse duration τ_p= 15ns. The repetition rate of the laser radiation was 1 Hz. Laser pulses with energy densities of F= 80mJ/cm^2 and F=25mJ/cm^2 were used in the presented experiments. The irradiation of the samples was performed using 2, 3 and 10 laser pulses. The potential anti-tumor effect *in vitro* of GNPs and laser treatment on HeLa and Hep-2 were studied by conventional tests. MTT assay was used for evaluation of cytotoxic effect after treatment. Apoptogenic effects on HeLa cancer cells were studied using fluorescence microscopy (AO/PI double staining method).

Results: Results obtained for HeLa cancer cell line were as follows: i) cells treated with GNPs or laser irradiation alone showed no significant difference from the control; ii) combination of GNPs and laser treatment showed highest cytotoxic effect when cells were incubated with gold nanoparticles with size 100 nm and irradiated with 3 pulses and F= 80mJ/cm^2. Lower results were obtained with GNPs with the same size and irradiation with 2 and 10 pulses and F=25mJ/cm^2; iii) when cells were treated with GNPs with size 40 nm, the best effect was observed after irradiation with 2 pulses and F=25mJ/cm^2. Data obtained for irradiation with 3 pulses and F= 80mJ/cm^2 and 10 pulses and F=25mJ/cm^2 were almost the same. For cancer cell line Hep-2 reduction of viable cells was observed, but the effect was considered to be very unsatisfactory.

Based on the results of MTT cytotoxicity assay and the slight effects observed on Hep-2 cancer cell line further investigations were performed on HeLa cells. Induction of apoptosis in treated with GNPs and irradiated cells was established using AO/PI double staining method. Well defined morphological features of apoptosis were observed in order to confirm the results from MTT cytotoxicity assay.

Based on the results of MTT-test and AO/PI double staining the combination of gold nanoparticles (both sizes) and laser treatment with different characteristics of the laser ray resulted in localized heating causing irreversible thermal cellular destruction.

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BO5. GENETIC POLYMORPHISMS OF GLUTATHIONE S-TRANSFERASES PI AND OMEGA IN RESPONSE TO CHEMOTHERAPY

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Pharmacogenetics trials basically favor efforts to study the origin and mechanisms of the individual drug responses, drug resistance, and adverse drug reactions. Individualization of anticancer therapy is attributed to genetic polymorphisms of drug metabolizing enzymes that associate with the risk of treatment failure or resulting in severe toxicity. Thus the pharmacogenetics of glutathione S-transferases (GSTs) as a determinant of cancer drug resistance, response of cancer patients to therapy, and patients survival were widely studied. Human GSTs are a family of phase II metabolism enzymes that have in addition a non-enzymatic function and are involved in stress response, apoptosis, as well as, in drug resistance. There is increasing evidence for the importance of GSTs as determinants of therapeutic response and in cytotoxicity of variety of chemotherapeutic drugs, such as platinum and arsenate drugs, and some novel drugs such as brostallicin (α- bromoacrylic). (1)
Drug resistance, intrinsic or acquired, is a major problem that limits the effectiveness of chemotherapy and is believed to cause treatment failure in over 90% of patients with metastatic cancer. The best-characterized mechanism of drug resistance of GSTs is via their ability to inactivate anticancer drugs. The formation of conjugates between the glutathione (GSH) and cispaltin, oxalplatin and carboplatin is a key step in their inactivation. High levels of GSH and GSTP1 class in particular, have been found in some tumor cells such as ovarian, resistant to platinum drugs. On contrary, low levels of GSTP1 have been correlated with improved overall survival following cisplatin treatment of other cancers as head and neck cancer. (2)

GSTs genotypes have been extensively studied for their potential role in drug resistance. Genetic variations in GSTP1 have been associated with changes in enzyme activity. Furthermore, there is evidence that GSTP1 105Val allele alone or in combination with GSTP1 114Val are much more active than the wild type alleles against cisplatin or carboplatin. (3) A recent study showed that GSTP1 alleles are protective against cisplatin and carboplatin therapy, and also protect against therapy-associated toxicities, such as, cisplatin-induced hearing impairment and oxalplatin-related cumulative neuropathy. (2,3) Expression patterns of GSTs in triple receptor negative breast cancer patients' cells showed association with the efficiency levels of carboplatin as part of polychemotherapy, as well. (4)

GSTO1 and GSTO2 catalize monomethyl arsenate reduction, the rate limiting reaction in arsenic biotransformation. Arsenic trioxide is used as a therapeutic agent in treatment of different neoplasms. Since patients treated with this drug can display striking toxicity, and the fact that some solid tumors were found to be resistant to the drug, studing the GSTO1 and GSTO2 pharmacogenetics could be usefull in understanding variation in response. (5,6)
Characterizing the pharmacological properties of novel drugs as brostallicin (a synthetic α-bromoacrylic, second generation DNA minor groove binder) showed that the GSH/GST system may play a peculiar role in determining the sensitivity of cells to this drug and these findings have potential value in cancer treatment. (1)
In conclusion, there is growing evidence for the role of GSTs via drug inactivation in the drug resistance in addition to other possible mechanisms as increased drug efflux and decreased

drug influx, alteration in drug target, processing of drug-induced damage, and evasion of apoptosis.

The involvement of GSTs enzymes in the drug responses should be followed in further studies on DNA and protein level.

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BP17. BRIEFLY ABOUT LITHIUM

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Lithium is an alkali metal element, number three in the periodic system. It is characterized with its low atomic weight and density, as well as its high reactivity. Lithium is one of the microelements, therefore biologically active. Although it is not of vital importance for the functioning of the human organism, lithium has a wide appliance in medicine and pharmacology, due to its ions favorable activity over many diseases, such as bipolar disease and schizophrenia. Many researches show good effects in the condition of bipolar patients, such as the activation of the growth of new gray matter in the brain, responsible for the control of the memories, emotions and concentration. Regardless of these interesting observations, the exact mechanism of the activity of lithium is so far remaining a puzzle, which is certainly making it very interesting and important to study.

Session C. Metal toxicity and cancerogenicity. Metals and environment.

Chairpersons:

Assoc. Prof. Anna Damianova, MD, PhD
Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences

Assoc. Prof Anastasia Daskalova, PhD
National Research Station for Wildlife Management, Biology and Pathology

Irena Andonova, PhD
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CO2. HEAVY METALS IN THE HOST-PARASITE SYSTEM OF HARES IN BULGARIA

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Our study was to assess the accumulation of heavy metals in hares (*Lepus europeaus* Pallas, 1778) and their endohelminths. The effect of parasites (cestodes or nematodes) on heavy metal concentrations in tissues of hares was established under industrial polluted field conditions. Contents of Cd, Pb, Co, Hg, Zn, Cu was determined in the liver, kidney and muscle in hares infected with *Trichostrongylus retortaeformis* **(**Nematoda) or with *Mosgovoyia pectinata* **(**Cestoda) in comparison to their helminths. Heavy metals were determined using an atomic absorption spectrophotometry. The bioaccumulation capacity of the helminthes was established. We found out that hosts infected with cestodes had lower levels of heavy metals Cd and Pb than hosts infected with nematodes. Levels of Co, Zn and Cu were similar in the hosts infected with cestodes or nematodes. Higher accumulation of Zn, Hg, Pb and Cd was found in the cestodes compared to their host and nematodes. The bioaccumulation factor is well expressed for Cd and Pb in the cestodes/muscle case. Taking into account all available data the model hare - *Mosgovoyia pectinata* presents the promising bioindication system to evaluate environmental heavy metals exposure in terrestrial habitats in field conditions.

CO2. HEAVY METALS AND THEIR HARMFUL EFECTS ON THE LIVING CELLS

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The biochemical mechanism of the affect of heavy metals and their salts on the cells in concentrations exceeding the physiological (permissible) concentrations in the organism involves structural and functional damages of the **biological membranes**. Their effect induces disturbances in the permeability of the membranes; changes in the membrane potential; ultrastructural damages of cellular organelle membranes. In many cases heavy metals suppress the function of the **electron transport chain/pathways** in the biomembranes resulting in decreased energy supply of the cells. Heavy metals such as Hg, Se, Ni, etc. affect **cell metabolism** inducing alterations of enzyme activity. They can inhibit the enzyme either directly; by interacting with the co-enzyme or with the substrate. In other cases heavy metals can stimulate enzyme activity. Biotransformation of the heavy metals and their compounds involves four types of enzyme reactions: oxidation**,** reduction, degradation (break down of molecules) and conjugation (combination of metal cations with different organic compounds in the cell). The effects of heavy metals (Pb, Cd, Cr, Ti, As) and their salts on the live cell manifest on **molecular and genetic level** as well. Due to their biochemical characteristics and great variety often their effect is not specific and induces severe alterations in cell genome exhibiting their **mutagenesis, cancerogenesis** and **terratogenesis**. Ontogenetic changes may occur in any stage from embryogenesis to senescence. The ontogenetic differentiation may not occur at the right time (heterochronia), not in the right place (heterotopia), may lead to the formation of abnormal structure (terratogenesis) or the entire ontogenesis may be damaged (embryotoxic effect).

Thorough morphological, biochemical studies and long-term monitoring are required for the elucidation of the mechanisms of heavy metals effects on molecular and cellular level and on the entire organism as well.

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CO3. IMPACT OF COBALT ON MALE FERTILITY

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Cobalt is a naturally occurring, relatively rare element of the earth's crust. It is an essential oligoelement for mammals in the form of cobalamin (vitamin B_{12}) [1]. There are 3 ways of mammalian exposure to cobalt – by food and drinks, by inhalation and by skin absorption. Cobalt does not accumulate in organism and is rapidly excreted in urine and hence the presence of cobalt in urine and blood is used as a biomarker for recent exposure to soluble cobalt [1]. Cobalt is important for the proper functioning of mammalian organism but exposure to high doses could exert adverse effects on various organs and tissues such as respiratory organs, skin, hematopoietic organs, myocardium, thyroid gland, reproductive organs [1]. Cobalt could also induce teratogenic and carcinogenic effects. It is shown to be number two of top five global allergens.

The present article is focused on the influence of cobalt on male reproductive organs and fertility. It is reported that cobalt exerts genotoxic effects on experimental animals by inducing a significant increase in the frequency of chromosomal aberrations in male gametes [2]. Another study reveals that cobalt treatment impairs male fertility demonstrated by significant increase in preimplantation losses and decrease in total and live births among untreated female animals that have been mated with treated males. Prenatal treatment with cobalt leads to abnormalities in urogenital system and testis descend is delayed. Significant decrease in epididymis and testis weight [4] is found as well as low sperm count and decreased motility [4], accompanied by morphological abnormalities of sperm [2]. On tissue level cobalt is shown to exert extension of interstitial blood capillaries, undulation of basal lamina and empty spaces throughout the seminiferous epithelium. Pathological changes in the seminiferous epithelium are manifested by vacuolation of Sertoli cells and formation of abnormal spermatid nuclei, followed by the presence of multinuclear cells and sloughing cells. Hypertrophy of Leydig cells is detected, as well [4]. All these changes lead to shrinkage of seminiferous epithelium although the volume of interstitium, diameter of seminiferous tubules and number of cell nuclei per area are significantly increased [3]. Interestingly serum testosterone levels are elevated while LH and FSH levels remain normal suggesting that cobalt interferes with local regulatory mechanisms of testosterone synthesis.

In conclusion cobalt application in adulthood affect male fertility but this negative effect could be neutralized after cease of treatment. However treatment during prenatal and/or postnatal development could lead to irreversible negative changes of male fertility. Therefore exposure to cobalt could be consider as a risk factor for male reproductive development and function and hence for male reproductive health.

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CP1. IRON AND ITS TOXIC EFFECTS

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Iron is a metallic chemical element with the symbol **Fe** (Latin: *ferrum*) and atomic number 26. Iron is a group 8 and period 4 element and is therefore classified as a transition metal.

Iron is essential to nearly all known organisms. In cells, iron is generally stored in the centre of metalloproteins, because "free" iron (which binds non-specifically to many cellular components) can catalyse production of toxic free radicals. Iron deficiency can lead to iron deficiency anemia.

In animals, plants, and fungi, iron is often the metal ion incorporated into the heme complex. Heme is an essential component of cytochrome proteins, which mediate redox reactions, and of oxygen carrier proteins such as hemoglobin, myoglobin, and leghemoglobin. Inorganic iron also contributes to redox reactions in the iron-sulfur clusters of many enzymes, such as nitrogenase (involved in the synthesis of ammonia from nitrogen and hydrogen) and hydrogenase. Non-heme iron proteins include the enzymes methane monooxygenase (oxidizes methane to methanol), ribonucleotide reductase (reduces ribose to deoxyribose; DNA biosynthesis), hemerythrins (oxygen transport and fixation in marine invertebrates) and purple acid phosphatase (hydrolysis of phosphate esters).

Iron distribution is heavily regulated in mammals, partly because iron has a high potential for biological toxicity. In mammals, dietary iron is absorbed from digestion of food in the duodenal portion of the gut. Specific iron transporters are present in epithelial cells that line the duodenum. A major component of this regulation is the protein transferrin, which binds iron absorbed from the duodenum and carries it in the blood to cells.

Toxic effect of iron overload on organ function. Iron overload induces organ damage in liver, heart, pancreas, thyroid, and the central nervous system. The main cause of this organ damage is due to the overproduction of ROS in the presence of excess iron.

Mechanism of iron toxicity. The production of ROS by iron is mainly through the Fenton reaction, which eventually forms hydroxyl radicals from superoxide or hydrogen peroxide. Among ROS, the hydroxyl radical is the most toxic fraction and it targets carbohydrate, protein, and nucleic acids. It is known thatthe reaction of hydroxyl radicals with the nucleic acid base 8-hydroxyguanine (8-OHG) is highly correlated with teratogenicity and carcinogenicity by oxidative stresses. Another powerful ROS showing similar reactivity as the hydroxyl radical is lipid hydroxyl-peroxide: ROOH. In iron overload, lipid peroxidative products such as malondialdehyde and 4-hydroxy-2-nonenal are increased, which form the radicals ROO-(alkyl oxyradical) and RO-(alkoxy radical). These lipid-based radicals possess longer half lives than hydroxyl radicals, and also have a stronger capacity for chronic cell toxicity and DNA damage.

Iron overload syndrome. Pathological conditions representing body iron overload are designated as iron overload syndromes, and iron deposition causes organ dysfunction including cell death, fibrosis, and carcinogenesis. Iron overload syndromes are classified as

genetic. Hereditary hemochromatosis is the most common genetic disorder in Western countries, and its clinicalmanifestation is systemic iron deposition mainly in liver, heart, brain, and endocrine organs. This organ damage is considered to be a result of tissue injuries by iron-induced oxidative stresses. In 1996, the causative gene was identified as HFE in the human chromosome 6, and approximately 85% of patients with hereditary hemochromatosis in Western counties have a homologous mutation of C282Y in their HFE gene. Thereafter, other genes such as hemojuvelin (HJV), TfR2, ferroportin, and hepcidin (HAMP) gene were identified. In spite of the lack of genetic background, iron overload is commonly observed as a secondary condition. The most common condition occurs in patients who require long-term blood transfusions due to severe anemias. This condition includes genetic disorders such as thalassemia and SCD, and anemia refractory to conventional treatments. In these patients, ineffective erythropoiesis and continuous accumulation of exogenous iron by transfusion are considered to be responsible for the iron overload. The resulting organ failures such as liver failure, cardiac failure, and severe diabetes mellitus affect patients' outcome. In addition to these classical conditions, there are many diseases that show mild iron deposition or dysregulation of body iron distribution. Such conditions include chronic hepatitis C, alcoholic liver disease, non-alcoholic steatohepatitis, and insulin resistance, and iron is an important cofactor that modifies these disease conditions. Furthermore, it is becoming clear that excess iron is also hazardous as it promotes atherosclerosis, carcinogenesis, diabetes, and other lifestyle-related disorders.

Iron is essential for the body, but extremely toxic when excess amounts are present. As the body has no active excretion pathways for iron, a continuous load of iron exceeding 1–2 mg/day will result in iron overload, and organ failures including liver and heart. The recent understanding of body iron metabolism at a molecular level enables us to elucidate the mechanism of iron toxicity more precisely. Improvement of patients' outcomes is becoming promising if a correct early diagnosis is made, and suitable management of these intractable conditions using iron chelation with high compliance is conducted.

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CP2. HEAVY METALS AND NEUROTOXICITIES EFFECTS

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Heavy metals are essential components of many neurobiological processes. Transition metals alter the redox state of the physical environment - catalyze redox reactions that are critical to the cellular respiration, chemical detoxification, metabolism, and even neurotransmitter synthesis.

Growing body of evidence suggests that metals may influence epigenetic phenomena which regulate expression of genes.

Neurotoxicity of Iron (Fe)

Iron is an indispensable element for a variety of cellular functions in metabolism, growth, and differentiation. Iron serves as a constituent of proteins, including ribonucleotide reductase and many other heme proteins, such as mitochondrial cytochromes, cytochrome P450 enzymes, and hemoglobin. Research on Fe and neurodevelopment has focused primarily on the effects of Fe deficiency anemia. There is evidence that excess Fe stores in pregnancy and newborns may be toxic. Several studies have reported about association between maternal hemoglobin or serum ferritin (SF) and low birth weight.

Ferritin is the major cellular iron storage protein. Iron regulates ferritin syntesis on both transcriptional and translational levels. It induces ferritin synthesis on translational level through regulation of iron regulatory proteins.

Excess Fe is known to be neurotoxic in adults, and the possibility that it may also produce health effects in pregnant women and newborns.

Neurotoxicity of Manganese (Mn)

Mn is essential nutrient and is required for many essential enzymatic reactions. On the other hand, mechanisms of Mn neurotoxicity are involved in increasing oxidative damage to neuronal cells. Excessive Mn intake is associated with several negative health outcomes: lethargy, tremor, and psychological and neurological disorders, resembling schizophrenia and Parkinson's disease. Numerous occupational studies document memory loss, anxiety, nervosity, impulsive-compulsive behaviors, psychotic experiences, fatigue, and sleep disturbances.

Exposure to environmental high levels of Mn has been associated with detrimental effect to neurodevelopment. Investigation of school-age children found out linear association between Pb concentration and intelligence quotient (IQ), when Mn concentration is above certain level (14 microg/L). This association is not observed if blood Mn concentration is with lower level.

Neurotoxicity of Copper and Zinc (Cu and Zn)

Neurotoxicity of Cu and Zn affects the nutritional deficiency and its effect on brain. Genetic diseases of excess Cu retention are well described and have significant neurologic sequelae. Wilson's disease is the most common of these diseases, and the presenting complaint for this genetic disorder frequently includes neurobehavioral changes resembling schizophrenia. Excess brain Cu is a common finding in neurodegenerative diseases such as Alzheimer's disease.

Zn deficiency has long been known to impact neurodevelopment adversely. Excess Zn, like excess Fe and Cu, is a common finding in neurodegenerative disease. Zn finger proteins are key transcriptional elements that regulate the cellular response to metal toxicity among other processes. Excess Zn is involved in the neuronal injury observed in cerebral ischemia, epilepsy, and brain trauma. The mechanisms by which Zn exerts its neurotoxicity include mitochondrial production of reactive oxygen species and the disruption of metabolic enzymes, ultimately leading to activation of apoptotic processes.

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CP3. HEAVY METALS EFFECT IN FETUS

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Several studies have established an association between DNA methylation and environmental metals, including Ni, Cd, Pb. Oxidative stress may be a unifying process to explain these findings across different metals. Metals are known to increase reactive oxygen species production in a catalytic fashion via redox cycling.

Fetal programming

The exposure to toxicants in early life may cause later life health effects. The observed phenomenon of "fetal origins of disease" suggests that early environmental exposures, such as metals, program later life gene expression. DNA sequence is static, genetic susceptibility from DNA sequence variation cannot explain the mechanisms by which prenatal or early childhood metal exposures impact cognition and behavior later in life. That mechanistic pathway is epigenetics. Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence. Such changes can have influences as profound as those exerted by mutations but, unlike mutations, are reversible and responsive to environmental influences. Although in the strictest sense epigenetics refers to changes in germ cell DNA methylation, the process of DNA methylation is critical more globally to cell differentiation and overall child development. Failure of DNA methylation systems in the brain leads to clinical syndromes such as mental retardation and autistic-like behaviors. Epigenetic modifications of regulatory DNA sequences in response to subtle variations in environmental conditions might be a critical source of variation in gene expression and function. If so, DNA methylation changes may serve as a process mediating the relationship between genome and environment throughout neurodevelopment. The effects of prenatal/early metal exposure on DNA methylation may program environmental exposures on the fixed genome, resulting in subtle but stable alterations in later life neurophenotypes.

Neurotoxicity is a common health endpoint for excess metal exposure. Finally, there is intriguing evidence that epigenetic phenomena may underlie observed effects of fetal or early life exposure and late onset of disease. Metals appear to alter DNA methylation, an epigenetic process by which gene expression is regulated. Further research in metals should include the role of epigenetics in determining long-term and late-onset health effects from metal exposure.

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CP4. SOIL POLLUTION WITH HEAVY METALS – A SURVEY AMID STUDENTS

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The major factor damaging the soil ecological equilibrium is pollution as a result of the humans' industrial activity and represents a particularly grave danger.

The purpose of this study is to evaluate the students' involvement in heavy metals pollution and conditions for human's health and the normal functioning of eco systems.

The research was undertaken amid 9-12 class students, participating in a club "Science". The latter exists in three Sofia schools within a project frame of Operative program "Development of human resources", financed by European Social Fund.

A specifically designed questionnaire was handed over at the end of one of the modules. As a result was noted that the students give a real evaluation of the circumstances for human health and for normal functioning of ecosystems from the pollution of the soil with heavy metals. The children shared that in the club they increased their awareness about this problem, influenced by: circulated Interdisciplinary teaching; achieved skills on team's work on cause dedication; participation in group-thesis discussions and arguing.

During the lessons the lectors encourage creative, original and resourceful thinking, which motivates additionally the children.

CP5. EVALUATION OF CHEMICAL CONTENT AND PHYSICO-CHEMICAL PARAMETERS IN SOME RILA MOUNTAIN RIVERS

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At present great number of river waters suffers from urban, industrial and agricultural pollution crossing different territories with anthropogenic influence. This influence creates high risk to the river environment. The assessment of discharges of certain dangerous

substances in the river ecosystem could be made by providing of comparison with their quantity in the clean river water from unpolluted mountain areas.

As an upper mountain part of Iskar river, the Moussalenska Bistrica river in Rila mountain has been investigated in this study. Monitoring of chemical content of different river components (water, sediment and mosses) in 2004, 2005 and 2006 has been carried out.

Simultaneously some physico-chemical parameters (pH, conductivity, TDS, salinity) for the tributaries of Iskar river also have been measured.

The results obtained show higher content of Mg in water and sediment in Moussalenska Bistrica river for 2004 and of Mg for 2005 in comparison with the other years.

The content of Mn and Zn in mosses is higher in 2005 compared with 2004 and 2006. The results of physico-chemical parameters are stable in the tributaries of Iskar river (Moussalenska Bistrica, Cherni and Beli Iskar).

Generally the results obtained show low levels of chemical elements in river components and stable physico-chemical parameters in the mountain tributaries of Iskar river.

CP6. CHEMICAL CONTENT OF MOUNTAIN ECOSYSTEM COMPONENTS AT DIFFERENT ALTITUDE IN RILA MOUNTAIN

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Monitoring and control of mountain areas are very important for the environmental assessment of the pollution. The chemical content of mountain components (soil, plants, etc.) vary at different altitude due to the local mineral specificy etc.
The amounts of atmospheric heavy metals (V, Mn, As, Zn etc.) are also considerable as some of them are incorporated by winds from other locations.

In this study different plants and soil from Rila mountain (Yastrebets - altitude 2360m and Borovets- 1350 m) have been investigated for their chemical content using AAS (Perkin Elmer 2 370A).

Some differences mainly in the soil chemical content have been obtained in the content of Mn, Cu and Zn which are higher in Borovets in 2007 in comparison with the results from 2008.

The comparison between the samples from two different altitudes (Borovets-1350 m and Yastrebets-2360 m) show also higher content of Mn, Cu, and Zn in the soil fromYastrebets. The results obtained show some differences in the chemical content in the soil from the two localities at different altitude.

CP7. EXPERIMENTAL STUDIES ON THE REMOVAL OF MERCURY FROM CONTAMINATED SOIL AND WATERS

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Mercury is a substance, which concentrates itself more and more in our natural environment, and can undergo easily in some organometallic mercury compounds much more toxic, such as methyl-mercury or tetraethyl mercury. Humans can absorb mercury through any mucous membrane and through the skin. As vapour, mercury can be inhaled, and ingested in foods such as fish, eggs, meat, and grain. In the body, mercury primarily affects the nervous system, the liver, or the kidneys, and induce poisoning symptoms such as: tremors, tunnel vision and loss of balance, slurred speech, and unpredictable emotions. In the present work, an experimental work regarding the optimal conditions of removing mercury from contamined soil and waters was performed. The study of mercury removal from contamined soil was performed using as extraction agent solutions 0.1M of EDTA. In order to determine the optimal conditions for removing mercury from soil sample, the dependence between the extracted mercury concentration and the liquid: solid ratio, shaking time, soil particle size and the type of extraction solutions was studied. The concentration of mercury was determined by atomic absorption spectrometry. The results indicated that EDTA is a worthy alternative to account for the extraction of mercury from contamined soils. The study of mercury removal from contamined waters, was carried-on using PUROLITE S920 ion exchange resins. The obtained results indicate the optimal parameters (the liquid: solid ratio, shaking time) for removing mercury from water solutions with known initial concentration of mercury, using the ion exchange resin PUROLITE S920.

CP8. REMOVAL OF HEAVY METAL IONS USING DYE ATTACHED TO COPOLYMER MICROBEADS AS SPECIFIC SORBENTS

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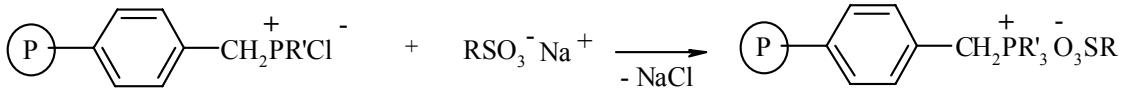
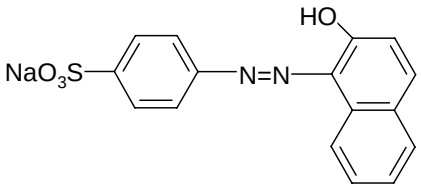
The aim of this work was the study of new specific sorbents for the removal of Cu^{2+}, Zn^{2+}, and Cr^{2+} ions from aqueous solutions. Styrene DVB functionalized with quaternary phosphonium groups microbeads was used as carrier matrix, and azo dye (AN) as the ligand.. Dye-attached to copolymer microbeads (SVB-AN) were characterized by UV-VIS and IR spectroscopy.



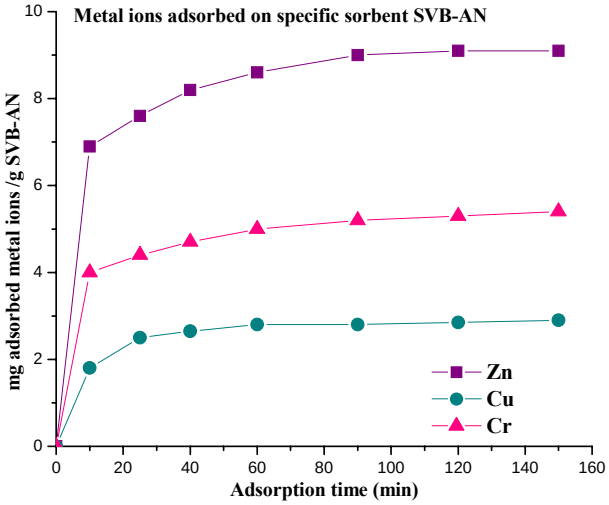
(SVB) (SVB-AN)

$RSO_3^- Na^+$:



(AN)

Dye-attached microbeads were used in the adsorption/desorption of the Cu^{2+}, Zn^{2+}, and Cr^{2+} ions, at 20°C. The maximum adsorption capacity decrease in order: $Zn^{2+} > Cr^{2+} > Cu^{2+}$ ions. The quantity of adsorbed metal ions on specific sorbent unit increased initialy with the metal ions concentration and after a definite period of time became constant reaching a plateau values. The maximum adsorption capacity of SVB-AN sorbent was: 9.4 (Zn^{2+}), 5.6 (Cr^{2+}), and 2.9 (Cu^{2+}) mg/ g sorbent. The desorbtion of metal ionw was studied by using 0.1 M HNO_3 (pH 1.0). Adsorption/desorption cycles showed the feasibility of repeated use of this novel sorbent system.



СР9. ЗА ТЕЖКИТЕ МЕТАЛИ, ВОДОРАСЛИТЕ И ОЩЕ НЕЩО

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Напредъкът на индустрията и все по-голямото желание на човека да модернизира технологиите, за да улесни живота си, довежда и до нежелани ефекти. Едно от най-често срещаните замърсявания е това на водата с тежки метали. То се дължи на замърсяването на атмосферата, на разпада и изветрянето на изкопаеми руди при добива на въглища, както и на дейността на промишлените предприятия. Повечето храни с консерванти, оцветители, стабилизатори, подсладители, както и генно модифицираните храни, влияят отрицателно на човешкия организъм. Може би трябва да изчакаме само няколко поколения, за да забележим резултата! Да изчакаме, разбира се, е в кавички. Защото фактът, че поради краткостта на човешкия живот няма да сме свидетели на случващото се, съвсем не означава, че трябва да стоим със скръстени ръце.

Твърде много е изписано за здравословното хранене респективно здравословните храни и начин на живот. Ето защо бих искал да обърна внимание на един друг проблем - замърсяване на водоемите с тежки метали. Тук е мястото да обърнем специално внимание на водораслите, които се очертават като екологични и икономически изгодни биоматериали, спомагащи за това пречистване – т.нар. биосорбция на тежки метали.

Тежките метали са устойчиви елемети, които при постъпване във водите се натрупват във живите организми. Повечето от тях са особено опасни, тъй като по пътя на хранителните вериги могат да постъпят в организма на човека и висшите животни и да предизвикат интоксикация.

Множество изследвания са посветени на адсорбция на тежки метали (Pb, Cd, Cu, Ni, Co, Zn, Co, Cr) от водорасли (кафяви, синьозелени и др.).

1. Кафяви водорасли

Поведението на кафявите водорасли при адсорбция на тежки метали е изучено от Thomas и колектив. Разгледан е механизмът на свързване на йоните на тежките метали, ролята на целулозната структура, на алгинатите и фукоидините, които влизат в състава на клетъчната стена.

В този ред на мисли бих искал да вметна, че възможността кафявите водорасли да абсорбират тежки метали, в никакъв случай не означава, че те са вредни като хранителна добавка (разбира се, ако са добити от воден басейн, в който металите и другите химични вещества са в допустимите граници). Нещо повече, нека не забравяме, че кафявите морски водорасли са богати на дефицитните в храната йод и селен, а също така калий, калций, мед, цинк, манган и др.

2. Сравнение по отношение брой свързващи активни центрове.

Направени са сравнения между три вида кафяви водорасли: *Sargassum hemiphyllum*, *Petalinia fascia*, *Colpomenia sinusa*, по отношение на активните им центрове. Най-голям брой свързващи активни центрове са установени при водораслите *Sargassum hemiphyllum* и *Petalinia fascia*.

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3. Синьозелени водорасли

По своята същност те са уникална комбинация от растения, животни и бактерии. Те съдържат една уникална съставка – *фикоцианинът* – синият пигмент, който спомага за освобождаването на организма от тежки метали и всякакви вредни вещества.

4. Поглъщане на тежки метали от микроводораслите

Установено е, че микроводораслите акумулират и концентрират големи количества тежки метали от средата на обитаване. Според Broda (1972) концентрацията на тези метали в клетките на *Chlorella* може да бъде 10^5 пъти по-висока, отколкото в окръжаващата ги среда. Към този феномен се наблюдава нарастващ интерес във връзка с възможността микроводораслите да участват в биологичното очистване и регулиране на промишленото замърсяване на околната среда. *Scenedesmus quadricauda* например натрупва в клетките си мед. *Pediastrum bouricanum* принадлежи към видовете, способни да детоксикират води, които са промишлено замърсени с живак.

5. Способност на микроводораслите да поглъщат голямо количество тежки метали за кратко време.

Известно е, че микроводораслите акумулират бързо оловото, дори когато неговата концентрация в средата е много ниска.

Количество на погълнато Pb (mg/dm^3) при различна температура.

Условия	Време, min							
	2	30	60	120	180	240	300	360
30C	6.33	6.50	6.57	6.58	7.00	7.61	7.85	7.66
20C		6.60				7.75		

Експериментални условия: Pb в средата 20 mg/dm^3; водораслова концентрация – 1mg сухо вещество в 1$см^2$.

Тази способност на микроводораслите може да бъде и нежелано явление, тъй като при масовото им култивиране това намалява качеството на биомасата. Особено нежелано е поглъщането на оловото. Допустимите граници за съдържание на олово в водорасловата биомаса или продуктите от нея, когато са предназначени за хранителни цели, е много ниска – 5 ppm.

6. Начин на приемане на тежки метали.

Статклиф (1964) и Hannerz (1968), са изказали предположението, че разликата в натрупването на олово при различните видове микроводорасли, зависи главно от свързващите свойства на клетъчната обвика. Марчуление и Нянишкене (1976) са установили, че при сладководните водорасли приемането на йони на живака, оловото и церия става по пътя на повърхностната адсорбция.

7. Влияние на тежките метали върху водораслите.

Попадналите в клетките на водораслите оловни йони, предизвикват физиологични и морфологични промени – поява на гигантски клетки с нетипична морфология. Чувствителни промени се наблюдава и в ултраструктурата на хлоропластите - намаляват фотосинтетичната фиксация на CO_2 (вероятно в резултат на влиянието на оловото върху ензимите на пентозофосфатния цикъл) и АТФ-синтезната активност.

Използвана литература:

- Диляна Вълчева Колева*, Христиана Николова Николова** - Биосорбция на тежки метали от води чрез зелени и кафяви водорасли (годишник на техническия университет – Варна 2007г.

CP10. PHYTOREMEDIATION. PHYTOEXTRACTION. EFFECT OF EDTA AND CITRIC ACID ON HEAVY METAL MOBILITY IN A CALCAREOUS SOIL AND HEAVY METAL UPTAKE BY *HELIANTHUS ANNUUS*

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Toxic metal pollution of waters and soils is a major environmental problem.Toxic metal remediation includes: (1) **Phytoextraction** - the utilization of plants to transport and concentrate metals from the soil into the harvestable parts of roots and above-ground shoots. Most traditional remediation methods do not provide acceptable solutions but phytoextraction

of metals is a cost-effective, sustainable approach that uses metal-accumulating plants to clean up these soils. (2) **Rhizofiltration** - the use of plant roots to remove toxic metals from polluted waters. Microbial populations are known to affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes, and therefore, have potential to enhance phytoremediation processes. (3) **Phytostabilization** - the use of plants to eliminate the bioavailability of toxic metals in soils. Pb is one of the most widespread and metal pollutants in soil. In general, metal hyperaccumulators are low biomass, slow growing plant species that are highly metal specific. For Pb, there are no hyperaccumulator plant species known to date. Although high biomass-yielding non-hyperaccumulator plants lack an inherent ability to accumulate unusual concentrations of Pb, EDTA has been proposed to enhance the metal concentration in above-ground harvestable plant parts. Calcareous soils with relatively high total metal contents are difficult to phytoremediate due to low soluble metal concentrations. Soil amendments such as EDTA have been suggested to increase heavy metal bioavailability and uptake in aboveground plant parts. Strong persistence of EDTA and risks of leaching of potentially toxic metals and essential nutrients have led to research on easily biodegradable soil amendments such as citric acid. The evolution of labile soil fractions of heavy metals over time was evaluated using water paste saturation extraction, extraction with 1 M NH_4OAc (soluble fraction) at pH 7 (exchangeable fraction), and extraction with 0.5 M NH_4OAc + 05 M HOAc + 0.02 M EDTA at pH 4.65(potentially bioavailable fraction). Both citric acid and EDTA produced a rapid initial increase in labile heavy metal fractions. Metal mobilization remained constant in time for soils treated with EDTA, but a strong exponential decrease of labile metal fractions was noted for soils treated with citric acid. High biomass producing plant species, such as *Helianthus annuus*, have potential for removing large amounts of trace metals. The low bioavailability of heavy metals in soils and the limited translocation of heavy metals to the shoots by most high biomass producing plant species limit the efficiency of the phytoextraction process. In one experiment plants were grown in a calcareous soil moderately contaminated with Cu, Pb, Zn, and Cd and treated with increasing concentrations of EDTA (0.1, 1, 3, 5, 7, and 10 mmol kg(-1) soil) or citric acid (0.01, 0.05, 0.25, 0.442, and 0.5 mol kg(-1) soil). Heavy metal concentrations in harvested shoots increased with EDTA concentration but the actual amount of phytoextracted heavy metals decreased at high EDTA concentrations, due to severe growth depression. The rapid mineralization of citric acid and the high buffering capacity of the soil made citric acid inefficient in increasing the phytoextracted amounts of heavy metals. Treatments with high concentrations resulted in a dissolution of the carbonates and compaction of the soil. These physicochemical changes caused growth depression of *Helianthus annuus*. EDTA and citric acid added before sowing of *Helianthus annuus* did not appear to be efficient amendments when phytoextraction of heavy metals from calcareous soils is considered. Due to environmental persistence of EDTA in combination with its strong chelating abilities, the scientific community is moving away from the use of EDTA in phytoextraction and is turning to less aggressive alternative strategies such as the use of organic acids or more degradable APCAs.

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CP11. MERCURY AND METHYLMERCURY CONTAMINATION IN THE IDRIJA RIVER AND THE GULF OF TRIESTE

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The presence of mercury in the river Idrijca (Slovenia) is mainly due to 500 years of mercury mining in this region. The Idrija Mine is a threat to the Idrija River and water bodies downstream including the Soca/Isonzo River and the Gulf of Trieste in the northern Adriatic Sea. The major source of inorganic mercury is still the River Soca (Isonzo) while the major source of methylmercury is the bottom sediment of the Gulf. To understand the cycling of mercury it is crucial to investigate the role of biota. A study was focusing on the accumulation and speciation of mercury in the lower levels of the food chain, namely filamentous algae, periphyton and macroinvertebrates. Mercury analysis in the biota and in water were performed during the spring, summer and autumn seasons at four locations on the river. Total (THg) and methyl mercury (MeHg) were measured. The highest THg concentrations in biota correlate well with THg levels in sediments and water. The level of MeHg was showing higher values at the most contaminated sites during the summer and autumn periods. The percentage of Hg as MeHg increases with the trophic level from water (0.1–0.8%), algae (0.5–1.3%), periphyton (1.6–8.8%) to macroinvertebrates (0.1–100%), which indicates active transformation, accumulation and magnification of mercury in the benthic organism of this heavily contaminated torrential river. A study conducted in June 1998 showed that Total Hg in the Idrija River increased >20-fold downstream of the mine from <3 to >60 ng liter (-1) with methyl mercury (MeHg) accounting for approximately 0.5%. Concentrations increased again downstream and into the estuary with MeHg accounting for nearly 1.5% of the total. Bacteria upstream of the mine did not contain mercury detoxification genes. Such genes were detected in bacteria collected downstream. Benthic macroinvertebrate diversity decreased downstream of the mine. Gulf waters near the river mouth contained up to 65 ng liter(-1) total Hg with approximately 0.05 ng liter(-1) MeHg. Gulf sediments near the river mouth contained 40 microgram g(-1) total Hg with MeHg concentrations of about 3 ng g(-1). Hg in sediment pore waters varied between 1 and 8 ng liter(-1), with MeHg accounting for up to 85%. Hg methylation and MeHg demethylation were active in Gulf sediments with highest activities near the surface. MeHg was degraded by an oxidative pathway with >97% C released from MeHg as CO(2). Macroinvertebrates and bacteria in the Idrija River responded to Hg stress, and high Hg levels persist into the Gulf. Increases in total Hg and MeHg in the estuary demonstrate the remobilization of Hg. Gulf sediments actively produce MeHg, which enters bottom waters and presumably the marine food chain. To demonstrate the power of precise isotope ratio measurements of Hg in environmental samples, sediments alongside the Idrijca River, the Soca/Isonzo River, and in the Gulf of Trieste were analyzed to determine the variation in Hg isotopic composition versus distance from the source. Sediments throughout the watershed of the Soca/Isonzo River to the Gulf of Trieste are dominated by Hg exported from the headwaters of the Idrijca River. Only the southern part of the gulf, outside the river plume, showed lower values of the isotopic composition. A simple binary mixing-model, could demonstrate that all samples in the study were a result of variable proportions of Hg originating from the Idrija region (progressively decreasing from >90% in the northern partto

<50% in the southern gulf) and from the Adriatic Sea. The tracking of mercury sources in natural systems using mercury stable isotope ratios is feasible. In the water column of the Gulf of Trieste higher bottom concentrations of dissolved Hg were probably due to remobilization from sediments, including resuspension and benthic recycling. The higher total MeHg in the bottom layer is the result of remobilization of MeHg from sediments including benthic fluxes. Temporal variability of mesozooplankton Hg and MeHg is the consequence of biomass and species variations and grazing behaviour.

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CP12. PHYTOEXTRACTION OF PB AND CD BY THE MEDITERRANEAN SALTBUSH: *ATRIPLEX HALIMUS* L. AND THE LEAVES OF *TAMARIX SMYRNENSIS* GROWING ON CONTAMINATED SALINE SOILS

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Phytoextraction is a strategy for the clean up of heavy metal contaminated soils. Phytoextraction depends upon the identification of suitable plant species that hyperaccumulate heavy metals. The Mediterranean saltbush *Atriplex halimus L.* is a C4 perennial native shrub of Mediterranean basin with an excellent tolerance to drought and salinity. Salinity changes the bioavailability of metals in soil and is a key factor in the translocation of metals from roots to the aerial parts of the plant. Three pot experiments were conducted under greenhouse conditions for a 10-week period with *A. halimus* grown in soil polluted with 20 ppm of Cd and/or 800 ppm of Pb and irrigated with three different salt solutions (0.0%, 0.5%, and 3.0% NaCl). Soil measurements for soil characterization were performed with the expiration of the first week of plant exposure to metals and NaCl, and at the end of the experimental period, chlorophyll content, leaf protein content, leaf specific activity of guaiacol peroxidase, shoot water content, biomass, and Cd and Pb content in the plant tissues were determined. Increasing salinity increases cadmium uptake by *A. halimus* L. while in the case of lead there was not a clear effect. *A. halimus* developed no visible signs of metal toxicity. Salt toxicity symptoms were observed in plants irrigated with 3% NaCl solutions. Chlorophyll content, leaf protein content, shoot water content and biomass were not negatively affected by the metals; instead, there was even an increase in the amount of photosynthetic pigments in plants treated with both metals and salinity. Salinity was found to have a positive effect on cadmium uptake by the plant. This may be related to a higher bioavailability of the metal in soil due to decreased Cd sorption on soil particles. On the other

hand, salinity did not influence in a clear way the uptake of Pb by the plant because of lead's limited mobility in soils and plant tissues. *Atriplex halimus* L. is a Pb- and Cd-tolerant plant. Phytoextraction by halophytes is a promising alternative for the remediation of heavy metal contaminated sites affected by salinity. Halophytes are also promising candidates for the removal of heavy metals from non-saline soils. The use of such plants can be potentially viewed as an alternative method for soil desalination where salt is removed from the soil instead of being washed downwards by water or other solutions. T. smyrnensis grown in polluted soil with 16ppm of cadmium and at three different salt concentrations (0.0, 0.5, 3.0% NaCl) for a 10-week period. It took place in an open-air area with natural light, at ambient temperature and humidity in an effort to keep the plants under conditions as similar as possible to those in the field. Care was taken not to let them be rained on. Temperature ranged from 19 to 50 degrees C with 33 and 21 degrees C being the average day and night temperature, respectively. Humidity ranged from 28% to 87% with a 13-14h photoperiod. The specific aims of this work are to investigate the accumulation of cadmium via root uptake at different saline conditions and cadmium excretion through salt glands on the surface of the leaves as a probable detoxification mechanism of the plant. Measurements of chlorophyll content, biomass, and shoot length are used to evaluate the potential of the plant for the removal of cadmium from contaminated saline and non-saline soils. Analysis of white salt crystals taken from glandular tissue confirmed the fact that this plant excretes cadmium through its salt glands on the surface of the leaves as a possible detoxification mechanism in order to resist metal toxicity. The presence of metals usually affects negatively the plant health, but T. smyrnensis developed no visible signs of metal toxicity, only salt toxicity symptoms were observed. Cadmium usually decreases the chlorophyll content in plants. The amount of photosynthetic pigments of T. smyrnensis was found not to be affected.

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CP13. THE INFLUENCE OF DEPTH ON MERCURY LEVELS IN PELAGIC FISHES AND FOREST FIRE'S INFLUENCE ON MERCURY ACCUMULATION BY FISHES VIA FOOD WEB RESTRUCTNG

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Mercury (Hg) is a trace element distributed throughout the earth's atmosphere, biosphere, and geosphere. With numerous redox states, mercury is readily transformed by a suite of physical and biologically mediated reactions, which results in global biogeochemical cycling and trace retention in plants and animals. Mercury enters food webs following abiotic or biotic (i.e., bacterially mediated) methylation after microbial uptake and subsequent consumer uptake, organic forms of mercury (primarily methylmercury - MeHg) readily bind to proteins and bioaccumulate in higher trophic level organisms.

There is a growing interest in determining mercury levels in the environment as well as in fish for human consumption. Methyl mercury is the most toxic and bioaccumulative form of mercury in food webs and it is the predominant chemical form making up 80 – 90%

of the total mercury present in fish muscle tissue. At the end of the food chain, fish and other organisms at the end of the food chain constitute the major source of MeHg in the human diet. It has been developed a cost-effective method for the analysis of methyl mercury in seawater fish muscle. The novelty of this method lies in the use of microwave-assisted extraction with acidic solution (HCl), addition of toluene, and subsequent extraction with cysteine acetate solution where only MeHg is present because of its affinity for cysteine groups. MeHg in cysteine phase and total mercury in the homogenate muscle tissue were determined using a direct Hg analyzer (DMA-80). Validation, precision, and accuracy of the method were evaluated and monitored with a tuna fish certified reference material (CRM 463) containing MeHg.

In a study by C. Anela Choya, which focuses on differences in ecology, depth of occurrence, and total mercury levels in 9 species of commercially important pelagic fish (Thunnus obesus, T. albacares, Katsuwonus pelamis, Xiphias gladius, Lampris guttatus, Coryphaena hippurus, Taractichthys steindachneri, Tetrapturus audax, and Lepidocybium flavobrunneum) from the central North Pacific Ocean, it is indicated that total mercury levels of predatory pelagic fishes and their prey increase with median depth of occurrence in the water column and mimic concentrations of dissolved organic mercury in seawater. It suggests that the mesopelagic habitat is a major entry point for mercury into marine food webs.

Another findings indicate that fishes from lakes in partially burned catchments contain greater mercury concentrations than fishes from reference catchments. Increased methyl mercury concentrations in fishes can result in serious health problems for consumers. The enhanced Hg accumulation is caused primarily by increased nutrient concentrations in the lake, which enhances productivity and restructures the food web through increased piscivory and consumption of Mysis. This restructuring results in increases to the trophic positions and Hg concentrations of fishes. Forest fire also causes a large short-term release of total Hg (THg) and MeHg to streams and the lake. This release initiates a small pulse of MeHg in invertebrates that contributes to enhanced Hg accumulation by fishes.

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CP14. METHYLMERCURY AND ITS INFLUENCE ON GREAT EGRETS (*ARDEA ALBUS*) AND AMERICAN KESTRELS (*FALCO SPARVERIUS*)

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In nature, animals can be used as bioindicators to provide an early warning of potential adverse, contaminant-related effects on organisms or populations themselves, on organisms or populations that prey upon them, and as sentinels for exposure and effects on humans.

Methylmercury is known to affect survival and reproduction in many species of birds and it is is a persistent pollutant that bioaccumulates in high-trophic-level predators.

A study made by the Department of Pathobiology, USA, shows the Histologic, neurologic, and immunologic effects of methylmercury in captive great egrets. Captive great egret (Ardea albus) nestlings were maintained as controls or were dosed with methylmercury chloride at low (0.5), and high doses (5 mg/kg, wet weight) in fish. When compared with controls, low dosed birds had lower packed cell volumes, dingy feathers, increased lymphocytic cuffing in a skin test, increased bone marrow cellularity, decreased bursal wall thickness, decreased thymic lobule size, fewer lymphoid aggregates in lung, increased perivascular edema in lung, and decreased phagocytized carbon in lung. High dosed birds had severe hematologic, neurologic, and histologic changes. The most severe lesions were in immune and nervous system tissues.It was made a comparison between the captive and wild birds and it as found that sublethal effects of mercury were detected at lower levels in captive than in wild birds. Conversely, thresholds for more severe changes (death, disease) occurred at lower concentrations in wild birds than in captive birds.

In another study, methylmercury accumulation in tissues showed effects on growth and appetite in captive great egrets. Captive great egret nestlings were maintained as controls or were dosed from 1- to 14-wk-old with 0.5 or 5 mg methylmercury chloride/kg wet weight in fish. Birds dosed with 5 mg/kg suffered from subacute toxicosis at wk 10-12. Blood concentrations of mercury increased more rapidly after 9 wk in all groups when feathers stopped growing. Total mercury accumulated in tissues in concentrations in the following order: growing scapular feathers > powderdown > mature scapular feathers > liver > kidney > blood > muscle > pancreas > brain > bile > fat > eye. After wk 9, appetite and weight index (weight/bill length) declined significantly in both dosed groups.

Kestrels have a long history of use in contaminant studies because they are relatively easily maintained in captivity and are closely related to peregrine falcons (F. peregrinus) that were once endangered by environmental contaminants (Wiemeyer and Lincer 1987).

In a study made by R. Bennett, J. French and team, American kestrels were fed meat diets containing 0, 3, 6 or 12 ppm (dry weight) methylmercury chloride. Birds fed the 12-ppm diet started to show signs of neurotoxicity after 26 days and all died in 39–49 days. . None of the birds fed the 3-ppm diet died or showed signs of toxicity. After 59 days of exposure, mercury concentrations in the liver, kidney, and blood of nonreproducing kestrels increased with increasing dietary concentration. Eggs averaged 8.3 and 18.1 ppm (wet weight) total mercury from birds fed 3- and 6-ppm diets, respectively. Feathers grown during mercury exposure contained high concentrations of mercury: Birds fed 3- and 6-ppm diets contained 275 and 542 ppm total mercury, respectively.

Those and many other studies are going to be used to improve assessments of mercury effects to predatory birds and might help explain the cause for declines in American kestrel populations in North America throughout its range.

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CP15. SUPPRESSED ADRENOCORTICAL RESPONSES AND THYROID HORMONE LEVELS IN BIRDS NEAR A MERCURY - CONTAMINATED RIVERS

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Endocrine assessment is a useful diagnostic tool in the detection of early or low-level responses to pollutants, responses that may precede more significant health problems. Although most of the studies on endocrine disruption or modulation have been focused on reproductive problems and behavioral abnormalities related to reproduction, contaminants may target other parts of the endocrine system more commonly than they disrupt processes involving gonadal steroids The hypothalamus–pituitary–adrenal (HPA) axis is an important system that regulates and integrates many physiologic functions in response to environmental stressors. Activation of the HPA axis during a stress response results in glucocorticoid secretion from the adrenal glands (mainly corticosterone in birds). This, in turn, initiates several important physiologic changes including effects on intermediary metabolism, growth, immune function, and inflammatory responses. Thyroid hormones— thyroxine (T4) and triiodothyronine (T3)—also play an important role in metabolism and exert profound effects on avian development (both differentiation and growth).

Much of the research on sublethal, adverse effects of mercury (Hg) has focused on impairment of neurological function and reproduction in fish and fish-eating vertebrates. In this study, they examine the associations between Hg and endocrine function (adrenocortical responses and plasma thyroid hormone concentrations) of insectivorous tree swallow nestlings adjacent to a Hg-contaminated river and nearby reference rivers in Virginia. Nestlings from the contaminated sites had blood Hg concentrations that exceeded those from the reference sites by more than an order of magnitude (354 +/- 22 vs 17 +/- 1 ppb wet weight). A regression of age and Hg concentrations suggested dietary Hg at the contaminated sites exceeded the nestlings' capacity to eliminate Hg through deposition into growing feathers. Although blood Hg concentrations among nestlings at the contaminated sites were lower than those typically associated with abnormal behavior or altered physiology in young birds, adrenocortical responses, plasma triiodothyronine, and thyroxin concentrations were suppressed, relative to reference levels, by the end of the nestling period. These results suggest that Hg may disrupt endocrine systems of terrestrial avian young and adverse effects of Hg on endocrine systems may be most evident once endocrine axes are fully developed.

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Session D. **Trace elements and minerals**

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DO1. EFFECTS OF SELENIUM SUPPLEMENTATION ON THE SERUM LIPID PEROXIDATION LEVEL AND ENOS-3 EXPRESSION IN AORTA AND MYOCARDIUM OF SPONTANEOUSLY HYPERTENSIVE RATS

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The selenium (Se)-dependent cellular glutathione peroxidase (GPx-1) is the most abundant intracellular isoform of GPx antioxidant enzyme family. It plays a major role in the control of reactive oxygen species (ROS) production which contributes to atherogenesis and hypertension pathogenesis and development. Tissue expression of selenoproteins is known to depend on daily Se intake. A diet containing 0,1 μg Se per gram of food is enough for normal growth and reproduction in mammals. Nitric oxide synthase (eNOS-3), a membrane-associated enzyme expressed in endothelial cells and cardiomyocytes, catalyzes the formation of nitric oxide (NO) from L-arginine. The NO affects the vessel tone and integrity, inhibits the smooth muscle cells migration and proliferation. It is responsible for the normal myocardium integrity and mediates the length-dependent increase in cardiac contraction force.

The aim of this study was to investigate the effects of Se supplementation on the serum lipid hydroperoxide (ROOH) production and eNOS-3 expression in aorta and myocardium of spontaneously hypertensive rats (SHR).

Sixteen 2-month old male SHR were divided into 2 groups: the first group (G1) received a dietary Se supplementation (0,4 μg Se/g of food) and the second group (G2) was fed in an adequate Se content diet (0,1 μg Se/g of food) for 8 weeks. The Se nutritional status was assessed by measuring whole blood GPx -1 activity using the "Ransel" kit of "Randox Laboratories LTD". The serum lipid hydroperoxide concentration was evaluated by the method of Yagi. The eNOS-3 expression was semi-quantativelly evaluated by light microscope examination using the kits and protocols of "Santa Cruz Biotechnology Inc". The statistical analysis was performed by the computer statistical program "Statgraphics v.4". F-test of Fisher (ANOVA), multiple range tests (LSD) and Chi- Square test were used.

The results showed an increased whole blood GPx -1 activity in G1 as compared to G2 ($p=0,03$). The lipid hydroperoxide concentration, however, was significantly reduced in the Se supplemented SHR ($p=0,026$). The eNOS-3 expression in G1 was significantly diminished in aorta ($p=0,01$) and myocardium ($p=0,02$) as compared to G2.

Various laboratories have reported upregulated cytosolic eNOS-3 expression and activity in mesenteric arteries from hypertensive rats, although eNOS-3 is a membrane-associated enzyme through posttranslational modifications. The physiological consequences of altered eNOS-3 compartmentalization are unknown. The cytosolic eNOS-3 could become uncoupled and that uncoupled eNOS can serve as a source of increased H_2O_2 production. In support of this hypothesis is the fact that small mesenteric arteries and aortic rings from hypertensive rats have been found to have significantly higher levels of basal H_2O_2 when compared with normotensive rats. It is well established that the endothelial dysfunction is mediated by reduced NO bioavailability and decreased activation of soluble guanylyl cyclase leading to blunted vasorelaxation. GPx-1 deficit due to low Se intake may directly induce an increased oxidative stress level thus leading to endothelial dysfunction. Data have been published that GPx-1 activity is lower in hypertensive subjects than in normotensive ones. The low GPx-1 activity reduces the bioactive NO concentration because of the altered eNOS-3 function and increased peroxinitrite formation.

In conclusion, Se supplementation has a positive effect in reducing the oxidative stress level and in increasing the NO bioavailability. Our results support the therapeutic benefit of Se administration in preventing and treating cardiovascular diseases which still remains insufficiently documented.

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DP1. COBALTABISDICARBOLLIDES AND THEIR INFLUENCE ON VIABILITY AND PROLIFERATION OF CULTURED TUMOR AND NON-TUMOR CELLS

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Boron, the fifth element in the periodic table has been found to be an essential mineral for animals and humans, and possess a broad spectrum of biological activities. Recently, there is an increasing interest in possible medicinal application of boron and boron compounds. The aim of this study was to evaluate the influence of cobaltabisdicarbollide [3,3'-Co(1,2-$C_2B_9H_{11})_2]^-$ (HCOS) and its derivatives [8-R-3,3'-Co(1,2-$C_2B_9H_{10}$)(1',2'-$C_2B_9H_{11}$)] $^-$ (R= CH_3COO- (209); -CH_3 (Me); -CH_2CH_3 (Et)) on viability and proliferation of cultured tumor cells. Permanent cell lines from human (carcinoma of the larynx Hep-2, breast adenocarcinoma MCF-7, glioblastoma multiforme 8 MG BA, lung cancer A542, cervical carcinoma HeLa and erythroleukemia K 562), mouse (myeloma P3U1), rat (virus-induced sarcoma LSR-SF-SR) and chicken (virus-induced hepatoma LSCC-SF-Mc29) were used as experimental models in our investigations. The effect of the compounds on cell viability and

proliferation was studied by MTT test, colony-forming method and neutral variant of single cell gel electrophoresis. The results revealed that the most pronounced cytotoxic and antiproliferative properties expressed Na[7], Na[6] and Na[3]), whereas Na[7] and Na[4] possessed the highest antimicrobial potential. The antimicrobial properties of the compounds examined were shown to be equal or even higher than those of the commercially available broad-spectrum antibiotic thiamphenicol that was used as a positive control. The cobaltabisdicarbollide H[**1**] was shown to express comparatively lower antibacterial and antifungal properties as compared to its derivatives.

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