

**PROCEEDINGS**  
**OF THE FIFTH WORKSHOP**  
**ON BIOLOGICAL ACTIVITY**  
**OF METALS AND METAL COMPOUNDS**



**WITH SATELLITE SYMPOSIUM**  
**ADVANCED MATERIALS IN BIOLOGY AND MEDICINE:**  
**CHALLENGES AND PERSPECTIVES**

**24-25 NOVEMBER, 2010**

**SOFIA, BULGARIA**

**Science is organized knowledge.  
Wisdom is organized life.**

**Immanuel Kant**



**P R O C E E D I N G S**  
**of THE FIFTH WORKSHOP**  
**“BIOLOGICAL ACTIVITY**  
**OF METALS AND METAL COMPOUNDS”**  
**with SATELLITE SYMPOSIUM**  
**“ADVANCED MATHERIALS IN BIOLOGY AND MEDICINE:**  
**CHALLENGES AND PERSPECTIVES”**

**24-25 NOVEMBER 2010, SOFIA, BULGARIA**

**Edited by**

**Radostina ALEXANDROVA, Reni KALFIN,**  
**Margarita GABRASHANSKA, Reneta TOSHKOVA,**  
**Stefka TEPAVITCHAROVA**

THE WORKSHOP AND THE SATELLITE SYMPOSIUM  
ARE ORGANIZED BY THE INSTITUTE OF EXPERIMENTAL MORPHOLOGY, PATHOLOGY  
AND ANTHROPOLOGY WITH MUSEUM (IEMPAM)  
UNDER THE AUSPICES OF  
THE BULGARIAN ACADEMY OF SCIENCES

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# THE PROGRAM OF THE WORKSHOP WITH THE SATELLITE SYMPOSIUM

Wednesday, November 24<sup>th</sup>

9.30 h-9.40 h **OPENING REMARKS**

## **Session A: METAL COMPOUNDS AS POTENTIAL THERAPEUTIC AGENTS**

**Chairpersons:**

**Assoc. Prof. Reneta Toshkova, MD, PhD**

*Institute of Experimental Morphology, Pathology and Anthropology with  
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*Institute of Neurobiology, Bulgarian Academy of Sciences*

**Secretary:**

**Elena Gardeva, DVM**

*Institute of Experimental Morphology, Pathology and Anthropology with  
Museum, Bulgarian Academy of Sciences*

**9.40-10.10 h PLATINUM-BASED CHEMOTHERAPY  
IN LUNG CANCER TREATMENT**

N. Miteva, K. Timcheva

**10.10-10.25 h PLATINUM, GOLD AND SILVER –  
THE NOBELS IN MEDICINE**

R. Alexandrova, O. Costisor, L. Patron

**10.25-10.40 h NEWLY SYNTHESIZED METAL COMPLEXES WITH  
THE MANNICH BASE TAMEN AND THEIR EFFECTS  
ON TUMOR CELL VIABILITY AND PROLIFERATION**

T. Zhivkova, R. Alexandrova, I. Vladov, D. Ivanov, M. Alexandrov,  
M. Georgieva, G. Miloshev, E. Mosoarca, R. Tudose, O. Costisor

**10.40-11.00 h COFFEE BREAK**

**11.00-11.15 h NEW METAL COMPLEXES OF URSODEOXYCHOLIC  
AND DEOXYCHOLIC ACIDS: INFLUENCE  
ON CELL VIABILITY AND PROLIFERATION**

L. Dyakova, R. Alexandrova, I. Vladov, D. Ivanov, M. Alexandrov, M.  
Georgieva, G. Miloshev, R. Kalfin, D. Culita, G. Marinescu, L. Patron

**11.15-11.30 h USING LIPOSOMES IN TRANSMEMBRANE  
TRANSPORT EXPERIMENTS**

Hr. Kolev, I. Pantcheva, M. Mitewa

**11.30-11.45 h AN ASSESSMENT OF THE APPLICATION OF  
MONENSIN AS AN ANTIDOTE FOR TREATMENT  
OF Cd (II) SUBACUTE INTOXICATION IN MICE**

Ju. Ivanova, Y.G. Gluhcheva, M. Mitewa

**11.45-12.00 h EFFECT OF MONENSIN ON VIABILITY AND  
PROLIFERATION OF VIRUS-TRANSFORMED  
TUMOR CELLS**

R. Alexandrova, T. Zhivkova, L. Dyakova, I. Pantcheva, M. Mitewa

**12.00-12.15 h PIROXICAM, MELOXICAM AND THEIR METAL  
COMPLEXES**

R. Alexandrova, T. Zhivkova, L. Dyakova, R. Kalfin, I. Vladov, M. Alexandrov,  
D. Ivanov, G. Marinescu, D.-C. Culita, L. Patron

**12.15 -12.30 h DISCUSSION**

**12.30 -13.30 h LUNCH TIME**

**Session B: ROLE OF METALS IN NORMAL AND PATHOLOGICAL  
PROCESSES**

**Chairpersons:**

**Assoc. Prof. Margarita Gabrashanska, DVM, PhD**

*Institute of Experimental Morphology, Pathology and Anthropology with  
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**Assoc. Prof. Anna Tolekova, MD, PhD**

*Faculty of Medicine, Trakia University, Stara Zagora*

**Secretary:**

**Dimitar Ivanov, PhD**

*Institute of Experimental Morphology, Pathology and Anthropology with  
Museum, Bulgarian Academy of Sciences*

**13.30-14.00 h CHANGES OF AORTIC WALL IN YOUNG  
SPONTANEOUSLY HYPERTENSIVE RATS  
UNDER DIET OF DIFFERENT SELENIUM CONTENT**

B. Ruseva, R. Ivanova, M. Mollova, A. Dimitrova, K. Tzachev

**14.00-14.30 h METALOEENZYMES AND OXIDATIVE STRESS  
IN HEMODIALYSIS PATIENTS**

Ts. Georgiev, P. Hadzhibozheva, V. Gadjeva, A. Zheleva, R. Iliev,  
P. Goicheva, V. Cholakov, A. Tolekova

**14.30-14.45 h EFFECT OF ZINC COMPOUND ON TRICHINELLOSIS  
IN MICE**

V. Nanev, S. Petkova, M. Gabrashanska, D. Ivanov

**14.45-15.05 h COFFEE BREAK**

**15.05-15.35 h СТРУКТУРЕН И ФУНКЦИОНАЛЕН АНАЛИЗ  
НА НОВ ТИП МЕТАЛОПРОТЕИН TS-PCHTP  
ОТ ПАРАЗИТНИЯ НЕМАТОД TRICHINELLA  
SPIRALIS**

Г. Радославов

**15.35-15.45 h СУПЕРОКСИД ДИСМУТАЗА**

Е. Стоилкова

**15.45-16.00 h IODINE FOR BODY USES AND DISEASE TRETMENT**

I. Mladenova

**16.00-16.20 h ESSENTIAL METALS Cu, Zn, Co, Ni - EDUCATIONAL  
BOOKLETS**

R. Alexandrova, R. Kalin

**16.20 – 17.00 h – POSTER SESSIONS A AND B**

**Thursday, November 25<sup>th</sup>**

**Session C: METAL TOXICITY AND CANCEROGENICITY.  
METALS AND ENVIRONMENT.**

**Chairpersons:**

**Assoc. Prof. Olga Baicheva, PhD**

*Institute of Experimental Morphology, Pathology and Anthropology with  
Museum, Bulgarian Academy of Sciences*

**Assoc. Prof. Anna Damianova, MD, PhD**

*Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of  
Sciences*

**Secretary:**

**Lora Dyakova**

*Institute of Neurobiology, Bulgarian Academy of Sciences*

**9.30-9.45 h INFLUENCE OF STEROID GLYCOSIDES ON MINERAL  
COMPOSITION OF THE PLANTS INFECTED BY ROOT-  
KNOT NEMATODE, MELOIDOGYNE ARENARIA**

O. Baicheva, A. Damianova, S.V. Zinovieva, Zh.V. Udalova, I. Vladov,  
I.S. Vasilieva, D. Salkova

**9.45-10.00 h ИЗСЛЕДВАНИЯ НА КОМПЛЕКСНОТО  
ВЪЗДЕЙСТВИЕ НА ГАМА-ОБЛЪЧВАНЕ  
И АДАПТОГЕН ВЪРХУ МИНЕРАЛНИЯ  
СЪСТАВ НА ИНВАЗИРАНИ С MELOIDOGYNE  
ARENARIA ДОМАТЕНИ РАСТЕНИЯ**

A. Damianova A., I. Vladov, O. Baicheva, S.V. Zinovieva, Zh.V. Udalova

**10.00-10.15 h CHEMICAL SPECIATION IN NATURAL  
AND BRINE SEA WATERS**

S. Tepavitcharova, T. Todorov, D. Rabadjieva, M. Dassenakis,  
V. Paraskevopoulou

**10.15-10.30 h HEAVY METALS CONTENT IN SOIL NEAR  
NON-FEROUS METALS PRODUCTION FACILITY  
AND DOMESTIC WASTES LANDFILL IN THE  
AREA OF KARDZHALI TOWN**

R. Chilingirova, J. Staykova, I. Velcheva, V. Naydenova

**10.30-10.45 h HEALTH STATUS OF THE STUDENTS  
IN KARDZHALI REGION**

J. Staykova

**10.45-11.00 h DISCUSSION**

**11.00-11.20 h COFFEE BREAK**

## **SATELLITE SYMPOSIUM**

### **ADVANCED MATERIALS IN BIOLOGY AND MEDICINE: CHALLENGES AND PERSPECTIVES**

#### **Session D**

##### **Chairpersons:**

**Assoc. Prof. Stefka Tepavitcharova, PhD**

*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences*

**Assist. Prof. Radostina Alexandrova, PhD**

*Institute of Experimental Morphology, Pathology and Anthropology with  
Museum, Bulgarian Academy of Sciences*

##### **Secretary:**

**Tania Zhivkova**

*Institute of Experimental Morphology, Pathology and Anthropology with  
Museum, Bulgarian Academy of Sciences*

**11.20-11.40 h NANOTECHNOLOGY AND THE CHALLENGES  
OF BRAIN TUMORS**

R. Alexandrova, L. Dyakova, T. Zhivkova, O. Alexandrov, M. Simeonova,  
R. Kalfin, C. Timcheva

**11.40-11.50 h ЗА НАНОТЕХНОЛОГИИТЕ И НАНОМЕДИЦИНАТА**

Д. Мартинов, Е. Стоилкова

**11.50-12.00 h AMORPHOUS CALCIUM PHOSPHATE FOR BI-PHASE  
CERAMICS PREPARATION**

D. Rabadjieva, S. Tepavitcharova, R. Gergulova, R. Titorenkova,  
E. Dyulgerova, O. Petrov



**12.00-12.10 h POROUS CALCIUM PHOSPHATES CERAMICS  
PROSPECTIVE AS BIOMATERIALS**

S. Tepavitcharova, D. Rabadjieva, K. Sezanova, R. Gergulova

**12.10-12.20 h ION MODIFIED CALCIUM PHOSPHATE CERAMICS**

D. Rabadjieva, S. Tepavitcharova, R. Gergulova, K. Sezanova,  
R. Titorenkova, E. Dyulgerova, O. Petrov

**12.20-12.30 h SYNTHESIS AND CHARACTERIZATION OF  
POLYMERIC HYDROGELS BASED ON NATURAL  
POLYSACCHARIDES AND GELATIN FOR  
BIOMINERALIZATION**

S. Shopova, E. Vassileva, D. Rabadjieva, S. Tepavicharova

**12.30 -13.30 h LUNCH TIME**

**12.30-13.30 h POSTER SESSIONS C AND D**

**13.30-13.40 h DOUBLE NETWORKS OF POLY(2-ACRYLAMIDO-2-  
METHYL-1-PROPANESULFONIC ACID)-  
POLYACRYLAMIDE AS MATRICES FOR CALCIUM  
PHOSPHATE CRYSTALLIZATION BIOMINERALIZATION**

L. Gencheva, M. Golyakova, E. Vassileva, D. Rabadjieva, S. Tepavitcharova

**13.40-13.50 h GELATIN MICRO- AND NANOCAPSULES AS A  
TEMPLATE FOR CALCIUM PHOSPHATES  
CRYSTALLIZATION**

M. Simeonov, E. Vassileva, D. Rabadjieva, S. Tepavicharova

**13.50-14.10 h BONE IMPLANTS – OLD PROBLEMS AND NEW  
PERSPECTIVEES**

R. Alexandrova, B. Andonova-Lilova, O. Alexandrov, T. Zhivkova,  
L. Dyakova, S. Tepavitcharova

**14.10-14.30 h AN ANIMAL MODEL IN RABBITS FOR  
BIOCOMPATIBILITY TESTING OF BIOMATERIALS  
IN BONES**

M. Gabrashanska, S. Tepavitcharova, M. Alexandrov, R. Alexandrova,  
V. Nanev, P. Dimitrov

**14.30-14.40 h BIOCHEMICAL INDICES IN RABBITS WITH  
BONE IMPLANTS**

M. Gabrashanska, S. Tepavitcharova, V. Nanev, P. Dimitrov

**14.40-14.50 h NANOMETRICAL BI-PHASE CALCIUM  
PHOSPHATE AND VITALITY OF CELL TEST**

R.Ilieva, E.Dyulgerova, R. Alexandrova, O.Petrov

**14.50-15.00 h FUNDAMENTAL APPLICATION OF TITANIUM  
AND TITANIUM ALLOY**

B. Andonova-Lilova

**15.00-15.20 h Coffee Break**

**15.20-15.50 h HONORARY LECTURE**

**DEFENSINS – THE NOVEL PUTATIVE  
ANTIRETROVIRAL TOOL**

Antcheva, N., A. Tossi, M. Vasileva, R. Gavazova, A.Hinkov, R.Argirova

**15.50-16.00 h DISCUSSION**

**16.00-16.10 h *CLOSING REMARKS***

## Session A. METAL COMPOUNDS AS POTENTIAL THERAPEUTIC AGENTS

### Chairpersons:

**Assoc. Prof. Reneta Toshkova, MD, PhD**

*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences*

**Assoc. Prof. Reni Kalfin, PhD**

*Institute of Neurobiology, Bulgarian Academy of Sciences*

### Secretary:

**Elena Gardeva, DVM**

*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences*

## AO1. PLATINUM-BASED CHEMOTHERAPY IN LUNG CANCER TREATMENT

**N. Miteva, K. Timcheva**

*Department of Chemotherapy, National Cancer Institute, Sofia*

Cisplatin, is a platinum-based chemotherapy drug used to treat various types of cancers, including small and non small cell lung cancer. It was the first member of a class of anti-cancer drugs which now also includes carboplatin and oxaliplatin. These platinum complexes react in vivo, binding to and causing crosslinking of DNA which ultimately triggers apoptosis Lung cancer represents the leading cause of cancer mortality worldwide, accounting for 1.2 million deaths each year. Improving survival in lung cancer is a major challenge for modern oncology. Because of the difficulties in significantly improving survival in locally advanced and metastatic non-small-cell lung cancer (NSCLC), treatment of early stages theoretically represent the most consistent possibility of modifying the outcome of NSCLC in terms of disease-free and overall survival. Just in the last 10 years adjuvant chemotherapy has become a recommended treatment for early stage NSCLC. Chemotherapy regimens showed a significant survival benefit for the treatment arm in stage II and III radically resected patients in adjuvant setting. In all studies cisplatin-based doublets were used, mainly cisplatin–gemcitabine, cisplatin-taxane or cisplatin-vinorelbine, with a cisplatin dose = 80 mg/m<sup>2</sup> and the survival benefit was consistent across the trials for stage II–IIIA. Neoadjuvant or preoperative chemotherapy is still considered. Preoperative cisplatin-based combination chemotherapy can be considered in patients with stage IIIA–N2 disease. Neoadjuvant regimens should be platinum based and at least three cycles of chemotherapy should be administered. In locally advanced NSCLC (stage III) adding platinum-containing chemotherapy with chest radiotherapy. Platinum-based combination chemotherapy prolongs survival, improves quality of life, and controls symptoms in patients with metastatic NSCLC. Limited-disease patients with SCLC should be treated with etoposide/platinum preferably etoposide/cisplatin, in combination with thoracic radiotherapy. Extensive disease patients should be treated with cisplatin or carboplatin in combination with etoposide at first line therapy.

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2. Bray FI, Weiderpass E. Lung cancer mortality trends in 36 European countries: secular trends and birth cohort patterns by sex and region 1970–2007. *Int J Cancer* 2009; 126: 1454–1466.
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6. Global cancer statistics. *CA Cancer J Clin* 2002;2005(55):74-108.

## **AO2. PLATINUM, GOLD AND SILVER – THE NOBELS IN MEDICINE**

**R. Alexandrova<sup>1</sup>, O. Costisor<sup>2</sup>, L. Patron<sup>3</sup>**

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## **AO3. NEWLY SYNTHESIZED METAL COMPLEXES WITH THE MANNICH BASE TAMEN AND THEIR EFFECTS ON TUMOR CELL VIABILITY AND PROLIFERATION**

**T. Zhivkova<sup>1</sup>, R. Alexandrova<sup>1</sup>, I. Vladov<sup>1</sup>, D. Ivanov<sup>1</sup>, M. Alexandrov<sup>1</sup>, M. Georgieva<sup>2</sup>, G. Miloshev<sup>2</sup>, E.-M. Mosoarca<sup>3</sup>, R. Tudose<sup>3</sup>, O. Costisor<sup>3</sup>**

<sup>1</sup>*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

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It was found in our previous investigations that some metal (CuI,II; CoII; NiII; FeII,III) complexes of the Mannich base N,N'-tetra-(antipyril-1-methyl)-1,2-diaminoethane (TAMEN) expressed antitumor properties in cultured human and animal cancer cells [1-3]. The aim of the present study was to evaluate the influence of 9 newly synthesized complexes of Cu(II), Co(II), Ni(II) and Mn (II) with TAMEN on cell viability and proliferation. Permanent cell lines established from cancers in human (MCF-7, 8 MGBA, A 549, HeLa, HepG2), rat (LSR-SF-SR) and chicken (LSCC-SF-Mc29) were used as experimental models. The nontumor human (Lep) cells were also included in some of our investigations. The study was performed by MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, colony-forming method, double staining with propidium iodide and acridin orange, method of Bradford, single cell gel electrophoresis in alkaline pH Comet assay). The results obtained revealed that: i) Applied at concentrations of 1-200 µg/ mL for 24-96 h the compounds examined decreased in a time- and concentration-dependent manner the viability and proliferation of the treated cells; ii) The copper complex Cu<sub>2</sub>(TAMEN)Br<sub>4</sub> demonstrated the most pronounced cytotoxic and antiproliferative properties; iii) Independently administered, the ligand TAMEN was found to be less active as compared to the metal complexes; iv) Among the cell lines used as model systems in the experiments, LSCC-SF-Mc29 chicken hepatoma cells were shown to be the most sensitive to the cytotoxic and cytostatic effects of the compounds tested; v)

The nontumor human Lep cells were not found to be more resistant to the influence of the compounds investigated than tumor human cell lines; vi) The metal complexes examined exhibited lower antitumor properties than the commercially available anticancer drugs cisplatin and 5-fluorouracil that were used as positive control in our study.

**Acknowledgements:** This study was supported by Grant CC D00-2-39/2009, National Science Fund, Bulgarian Ministry of Education and Science.

#### Referemnces:

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2. Alexandrova R.I., Rashkova G., Slavov S., Nikolova E., Kirilova M., Miloshev G., Mosoarca E.M., Tudose R., Costisor O. *5<sup>th</sup> International Symposium on Trace Elements in Human: New Perspectives. October 13-15 October, 2005, Athens, Greece. Proceeding Book*, pp. 242-250. Cytotoxic and antiproliferative effects in vitro of iron complexes with Mannich type ligands.
3. Alexandrova R., Rashkova G., Popova T., Tudose R., Mosoarca E.M., Slavov S., Costisor O. *Acta Morphol. Anthropol.*, 2006, 11, 60-85.

### AO4. NEW METAL COMPLEXES OF URSODEOXYCHOLIC AND DEOXYCHOLIC ACIDS: INFLUENCE ON CELL VIAVILITY AND PROLIFERATION

L. Dyakova<sup>1</sup>, R. Alexandrova<sup>2</sup>, I. Vladov<sup>2</sup>, D. Ivanov<sup>2</sup>, M. Alexandrov<sup>2</sup>, M. Georgieva<sup>3</sup>, G. Miloshev<sup>3</sup>, R. Kalfin<sup>1</sup>, D.-C. Culita<sup>4</sup>, G. Marinescu<sup>4</sup>, L. Patron<sup>4</sup>

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Bile acids (BAs) are a group of molecular species of acidic steroids with peculiar physical chemical and biological characteristics. Primary BAs (such as cholic and chenodeoxycholic) are directly synthesized from cholesterol by hepatocytes, by the addition of hydroxyl groups and the oxidation of its side chain to form more water soluble end product. The secondary bile acids (such as deoxycholic, lithocholic, ursodeoxycholic) are generated in the intestine by bacterial biotransformation of primary BAs [1,2].

In recent years steroidal structures have become increasingly important in a number of fields such as pharmacology, medicinal chemistry, biomimetic, supramolecular chemistry and also in nanotechnology. There are well known pharmacological applications of bile acids and their derivatives, including their use in the treatment of liver diseases, in dissolution of cholesterol gallstones, as well as their potential to act as carriers of liver specific drugs and cholesterol level lowering agents [3]. At the same time, there are multiple epidemiologic data and scientific reports suggesting the role of bile acids in pathogenesis of human malignancies, especially those of the gastrointestinal tract [4]. In contrast, other studies have shown that bile acids exert cytostatic and cytotoxic effects in several human cell lines established from cancers of the breast [5], ovary [6], uterine cervix [7], prostate [8], liver [9] etc. Bile acids were also reported to inhibit angiogenesis in human hepatocellular carcinoma cells [10] and induce differentiation in human acute promyelocytoc leukemia cells [11]. The sum of these observations point to the necessity of further investigations to clarify better the biological activities of bile acids.

In this study the data about the effects of the secondary bile acids – ursodeoxycholic (UDCA) and deoxycholic (DCA) acids and their complexes with CuII, CoII, ZnII and NiII on viability and proliferation of cultured human and animal tumor and nontumor cells will be presented.

**Acknowledgments:** This work was supported by Grant DO-02-39/2009 from the National Science Fund, Sofia, Bulgaria.

## References:

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## AP1. BIOLOGICAL ACTIVITY OF NOVEL COMPOUNDS OBTAINED ON INTERACTION OF 1,10-PHENANTHROLINE WITH ALKALINE EARTH METAL IONS, PALLADIUM (II) AND NaBF<sub>4</sub>

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Complexes of alkaline earth metal ions and palladium (II) with 1,10-phenanthroline as well as the protonated forms of the trimer and dimer of 1,10-phenanthroline were synthesized. Their in vitro antibacterial activity and in vivo acute toxicity were determined. All compounds showed a well expressed activity against the Gram-negative strain *E. coli* and a weaker activity against *S. aureus*. Only the compound (phen)<sub>2</sub>(H<sup>+</sup>)(BF<sub>4</sub><sup>-</sup>) revealed a very slight activity against *P. aeruginosa*. The palladium complex Pd(phen)<sub>2</sub>(H<sub>2</sub>O)(NO<sub>3</sub>)<sub>2</sub> showed the highest activity against the *E. coli* strain. The compounds (phen)<sub>3</sub>(H<sup>+</sup>)<sub>2</sub>(NO<sub>3</sub><sup>-</sup>)<sub>2</sub> and (phen)<sub>2</sub>(H<sup>+</sup>)(BF<sub>4</sub><sup>-</sup>) do not contain a metal ion, but nevertheless revealed an activity against both *E. coli* and *P. aeruginosa*. It is noteworthy that the described substances are not related to any known group of antibiotics and do not show acute toxicity when administered intraperitoneally in rats at doses up to 10 mg.

## AO5. USING LIPOSOMES IN TRANSMEMBRANE TRANSPORT EXPERIMENTS

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Monensin is an antibiotic produced by *Streptomyces cyananmonensis* bacterial strain. Unlike quasi-ionophores that form transmembrane channels permeable for metal ions, monensin forms neutral metal complexes that are free to enter and leave the phospholipid bilayer. Monensin is well known for its complexation affinity towards single charged ions like  $K^+$ ,  $Na^+$ ,  $Tl^+$  and  $Ag^+$ .

Our laboratory had been working on synthesizing divalent metal complexes of polyether ionophorous antibiotics. We present four metal complexes of monensin and sodium monensin - two with  $Co^{2+}$  and two with  $Mn^{2+}$ . X-ray structures obtained from grown monocrystals allowed us to find the relationship between their structure and properties in the experiments that followed.

The recent tendency in experimenting with biophysical objects as phospholipid membranes, mono- and bilayers calls for the development of new experimental procedures. Our laboratory has developed analytical techniques based on luminescence to study processes occurring in the phase boundary between the aqueous and the lipid phase in the form of phospholipid vesicles. The greater performance and sensitivity of luminescent methods allows very precise experiments to be carried out.

We used liposomes filled with fluorescent chelator to study the rate by which divalent metal complexes of monensin diffuse through the phospholipid bilayer. Those experiments are more plausible when thinking of resorption and membrane permeability, because they reflect not only the partition coefficient of the compounds but also the lipid sorption-desorption kinetics.

Because the membrane's viscosity is crucial for many of its properties we made some experiments to monitor this factor during the diffusion experiments. Using the hydrophobic micro viscosity probe auramine O, we were able to monitor the change in lipid viscosity throughout the penetration process during the diffusion. These results together with the ones obtained with the fluorescent chelator could be used to interpret the microbiological activity of the four compounds.

**Acknowledgements:** The National Science Fund (DO-02-84/2008) supports this work.

## **AO6. AN ASSESSMENT OF THE APPLICATION OF MONENSIN AS AN ANTIDOTE FOR TREATMENT OF Cd (II) SUBACUTE INTOXICATION IN MICE**

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Cadmium (Cd) is one of the most toxic metals emitted in our environment. Due to its very long biological half life it accumulates in living organisms causing severe organ and tissues damages [1]. Chronic exposure to Cd results in renal dysfunction, anemia, hepatic dysfunction, and osteotoxicities [2]. Cadmium is a potent human carcinogen and has been associated with cancers of the lung, prostate, pancreas, and kidney [2].

Chelation therapy is applied for treatment of acute and chronic heavy metal intoxication. It is based on a reaction of complex formation of metal ions with chelating agents. These ligands promote the excretion and subsequent depletion of the metal ions in living organisms [3]. Most of the chelating compounds applied in the chelation therapy so far are hydrophilic and their ability to access intracellular metal is weak. The development of new chelators as well as new strategies in chelation therapy to address this drawback is required [2,3].

Recently, it has been demonstrated that the polyether ionophore antibiotic Monensin could be applied as an antidote for treatment of lead intoxication in rats based on the complex formation with the toxic metal ion. In contrast to other chelators as EDTA and DMSA, Monensin is hydrophobic and significantly reduces lead levels in bone and brain without affecting Cu(II) and Zn(II) homeostasis [4,5]. In order to assess the application of Monensin as a potential antidote for treatment of intoxication with other toxic metals detail study on the complexation of this antibiotic

with toxic metal ions (Cd, Hg, As, etc) is needed. Herein we report the structure of Cd (II) complex with Monensic acid and present results from studies with animal models as an initial attempt to assess the potential application of Monensic acid as an antidote for treatment of subchronic cadmium intoxication.

**Acknowledgements:** The financial support of this work by University Fund for Science Research (005/2010) is gratefully acknowledged.

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## AO7. EFFECT OF MONENSIN ON VIABILITY AND PROLIFERATION OF VIRUS-TRANSFORMED TUMOR CELLS

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Monensin, an antibiotic produced by fermentation of a strain of *Streptomyces cinnamonensis*, is a carboxylic Na<sup>+</sup> ionophore used worldwide in veterinary medicine as a growth promoter in beef cattle and feed additive against coccidiosis in chickens and pigs. It has long been considered that activation of the Na<sup>+</sup>/H<sup>+</sup> antiporter and the resulting changes in pH participate in many cellular functions such as proliferation, differentiation and apoptosis. In particular, monensin is known to transport 1 molecule of Na<sup>+</sup> into cell per molecule of H<sup>+</sup> transported out of cells, resulting in intracellular alkalization. In 1995 Zhu and Loh reported that monensin caused apoptosis as well as intracellular alkalization in HL-60 human leukemia cells. It has recently been published that this compound can potently inhibit growth and induces apoptosis in various cell lines established from acute myelogenous leukemia, lymphoma, myeloma, renal cell carcinoma and cancers of the colon, breast and uterine cervix. In addition, monensin inhibited in vivo the growth of murine leukemia WEHI-3BD cells in BALB/c mice.

In order to broaden the histologic types of tumors studied, in this study we evaluate the effect of monensin on viability and proliferation of virus-transformed tumor cells.

We decided to use as model systems the cell lines LSCC-SF-Mc29 (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29) and LSR-SF-SR (Transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin) because of the following main reasons: i) according to the literature available, the effects of monensin on viability and proliferation of retrovirus-transformed cells have not been evaluated yet; ii) the cells of both lines carry specific viral oncogenes – v myc (in the case of LSCC-SF-Mc29) and v-src (in the case of LSR-SF-SR). It is well known that *myc* (*c-myc*, *L-myc*, *N-myc*) and *src* proto oncogenes are involved in pathogenesis of a wide variety of malignancies in humans; iii) these cell lines were successfully used in our previous investigations to evaluate the putative antitumor activity of alkaloids and metal complexes with various ligands. The experiments performed by MTT test,



colony forming assay, method of Bradford and double staining with acridin orange and propidium iodide revealed that monensin (applied at concentrations of 1 – 25 µg/ml for 24h, 48h and 72h) decreased significantly the viability and proliferation of the cultured chicken hepatoma and rat sarcoma cells in a time- and concentration-dependent manner.

**Acknowledgements:** The present research is supported by the National Science Fund in Bulgaria (Grant DO-02-84/2009).

## **AO8. PIROXICAM, MELOXICAM AND THEIR METAL COMPLEXS**

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Zn (II), Cu (II), Co (II) and Ni (II) complexes with two non-steroidal anti-inflammatory drugs (piroxicam, a non-selective COX inhibitor, and meloxicam - selective COX-2 inhibitor) were synthesized and their physical and chemical characteristics were determined. The cytotoxic and antiproliferative properties of these compounds were examined using a panel of 7 tumor cell lines: chicken LSCC-SF-Mc29 (chicken hepatoma induced by the myelocytomatosis virus M 29), rat LSR-SF-SR (sarcoma induced by Rous sarcoma virus stain Schmidt Ruppini) and human 8 MGBA (glioblastoma multiforme), A549 (non – small cell lung cancer), MCF-7 (breast cancer), HepG2 (hepatoma), HeLa (cervical carcinoma), HT 29 (colon cancer).

The results obtained by MTT test, neutral red uptake cytotoxicity assay and crystal violet staining revealed that when applied at concentrations of 10 - 200 µg/mg for 24-120 h, the non-steroidal anti-inflammatory drugs examined and their metal complexes decreased in a time- and concentration- dependent manner the viability of the treated cells. The compounds investigated were also found to inhibit the colony-forming ability of tumor cells and to induce cytopathological changes. In our experiments we found that the metal complexes examined possess more pronounced cytotoxic and antiproliferative activity as compared to the ligands – meloxicam and piroxicam. The most pronounced cytotoxic and cytostatic properties were demonstrated by the Ni(II) complex of meloxicam.

**Acknowledgements:** This study was supported by Grant DO-02-39/2009, National Science Fund, Bulgarian Ministry of Education and Science.

## **AP2. ANTITUMOR ACTIVITY OF GLYCOSYLATED MOLLUSCAN HEMOCYANINS IN RATS WITH GUERIN ASCITES TUMOR**

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As observed in most molluscan hemocyanins, high-mannose type glycans were identified in hemocyanins from *Rapana venosa* (RvH), *Helix lucorum* (HIH) and keyhole limpet (*Megatoura crenulata*). In addition, a glycan with a branching structure containing xylose, fucose and terminal methyl hexose was identified in  $\beta$ -HIH. We have examined the immuno-adjuvant properties of hemocyanins, their derivatives and conjugates associated with the cell mediated immunity in experimental tumor-bearing animals with ascites tumor of Guerin. After immunization of the animals with the experimental vaccine preparations, the highest values of splenic lymphocytes were observed in groups immunized with the conjugates RvH-TAg,  $\beta$ -HIH-TAg and KLH-TAg (42,3%; 40,8% and 40,58%, respectively) than with the native hemocyanins (36,5%; 35,1% and 32,4%, respectively). The immunization of rats with the hemocyanins  $\beta$ -HIH, RvH and KLH and their conjugates, prolonged the median survival time of tumor-bearing animals compared with non-immunized animals (39, 33, 31 and 7 days, respectively). Our results are the first demonstration of the antitumor effect of hemocyanins besides the investigations with keyhole limpet and *C. concholepas* hemocyanin. It was established that both hemocyanins  $\beta$ -HIH and RvH activate the immune system of the experimental animals and therefore could be a good alternative for KLH. For this purpose they could be included into the composition of non-specific anti-tumor vaccines to enhance their effectiveness.

**Acknowledgments:** This work was financially supported by the research grants: BG051PO001-3.3.04/46 from European Social Fund; Operational program "Development of human resources" 2007-2013 and by the Bulgarian National Science (UVL-301).

### AP3. ANTICANCER AND ANTIOXIDANT ACTIVITY OF METALS

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Although our cells have very efficient antioxidant defence systems to neutralise harmful free radicals, these systems are not 100% efficient, and over time biochemical damage gradually accumulates, leading to a reduction in cellular function. Most scientists now believe that accumulated cellular free radical damage lies at the heart of the ageing process and many degenerative diseases such as cancer, autoimmune diseases and Alzheimer's disease.

The most famous antioxidant elements are potassium, calcium, copper, zinc, selenium and magnesium. Potassium can have a positive effect in lowering blood pressure. Selenium has antioxidant properties, rendering harmful free radicals safe and is therefore implicated as an anti-cancer agent. Copper is a vital mineral required to make collagen. Lack of copper is linked with osteoporosis and heart disease. Phosphorus is the companion nutrient to calcium. Antioxidants have been shown in animal studies to also not only prevent strokes, but to hasten the recovery from strokes. The antioxidant studies identify further brain benefit, crediting high antioxidant diets with a wide range of neurological benefits. Improved memory and learning were identified.

The development of metal complexes with anticancer activity has had an enormous impact on cancer chemotherapy. The discovery of cisplatin in the 1960's represented a landmark achievement. Despite the fact that cisplatin has achieved significant clinical benefit for several types of solid tumors, its effectiveness has been hampered by toxic side effects and tumor resistance that often leads to the occurrence of secondary malignancies. However, discovery and use of cisplatin have encouraged investigators to search for and develop novel non platinum-containing metal species with superior anti-cancer activity and low side effects. As examples, gallium salts and gold complexes have been evaluated in phase I and phase II trials. Copper-chelating compounds have also shown promising results in both preclinical and clinical studies.

## AP4. THE ANTIVIRAL ACTIVITY OF METAL COMPOUNDS

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Viruses are the smallest infectious agents containing of a single nucleic acid (RNA or DNA) encased in a protein shell, which may be covered with a lipid containing membrane. More than 300 viruses are known to be pathogens in humans and animals producing a variety of syndromes. Viruses are still the most common agents of all human ailments.

The identification of new antiviral drugs is a challenging endeavor that aims to strike a balance between potent antiviral activity and minimal toxicity against host tissues.

Metals have been applied for medicinal purposes since ancient times. Thus, the Chinese were using elemental gold for the treatment of diseases, a practice known as chrysotherapy, as far as 2500 BC. The aim of the study presented here was to summarize data published about antiviral activity of metals and metal compounds.

**Copper.** It is well known that bacteria and other microorganisms have different mechanisms to deal with excess metal like copper. At the same time, viruses do not possess mechanisms to tolerate excess metal ions, making them extremely susceptible to copper ions. Copper has been shown to inactivate a variety of enveloped and nonenveloped viruses, such as bacteriophages, poliovirus, herpes simplex virus (HSV) and human immunodeficiency virus (HIV-1).

Sagripanti and Lightfoote reported in 1996 that HIV-1 was inactivated by cupric ions when the virus is free in solutions and also 3 h after cell infection. Subsequently, it was showed that copper ions inactivate the HIV-1 protease, which is an essential protein for replication of the virus. In addition, copper ions may cause nonspecific damage to HIV virions by damaging their envelope phospholipids and denaturing the virus nucleic acids by binding to and/or disordering helical structures and/or by cross-linking between and within the nucleic acid strands. Copper(2+) ions suppress the infectivity of avian influenza virus in MDCK cells at lower concentrations at which neither neuraminidase (NA) nor hemagglutination inhibition occurs. Electron microscopic analysis revealed morphological abnormalities of the Cu(2+)-treated H9N2 virus. Additional studies should be undertaken to clarify the mechanism underlying the antiviral effect of copper ions on influenza virus.

**Zinc.** Zinc salts have been shown to have *in vitro* activity against a variety of pathogens, including HIV, HSV, etc. Zinc salts (zinc lactate and zinc gluconate) have been found to inactivate clinical isolates of herpes simplex virus *in vitro*.

Zinc ionophores pyrithione and hinokitiol have been reported to inhibit picornavirus replication by interfering with proper processing of the viral polyprotein.

Zinc supplementation has been shown to contribute to inhibition of liver fibrosis and improvement in hepatic encephalopathy. Polaprezinc (a complex of zinc and L-carnosine) exerts an anti-inflammatory effect on the liver in patients with hepatitis C virus (HCV)-related chronic liver disease by reducing iron overload. Zinc is a negative regulator of HCV replication in genome length HCV RNA-replicating cells (but not in the subgenomic HCV replicon system). The prophylactic use of ZnAL42 against avian influenza H5N1 or H1N1 virus infection in mice has also been reported.

**Cobalt (III)** ligand complexes have been found to possess antiviral properties. In 1998, Epstein and coworkers reported that the cobalt complex CTC-96 was effective in the treatment of epithelial herpetic keratitis, one of the major causes of blindness in industrial nations. Studies using the CTC class of drugs were performed using a rabbit eye model infected with HSV-1 and all complexes inhibited HSV-1 replicatiopn *in vitro* with as little as 5 µg/mL required for strong antiviral activity. Although the mechanism of action of the CTC class of complexes has not been completely elucidated, it has been suggested that the molecular target is the herpes virus

maturational protease, a serine protease containing large amounts of the amino acid histidine. There is evidence that CTC-96 inhibits membrane fusion events preventing virus entry as well as plaque formation by VSV (vesicular stomatitis virus) and VZV (varicella zoster virus). The activity of CTC against adenovirus in a cell culture model as well as against keratoconjunctivitis in a rabbit model was also reported.

Hexamminecobalt (III) chloride  $[\text{Co}(\text{NH}_3)_6]\text{Cl}_4$  is a commercially available compound that significantly inhibited Sindbis virus replication in baby hamster kidney (BHK) cells in a concentration- and time-dependent manner.

**Nickel.** Nickel(II)-xylylbicyclam is a potent anti-HIV agent and binds strongly to the CXCR4 co-receptor.

**Bismuth.** A series of bismuth complex were synthesized and characterized, and most of them exhibited inhibition against the severe acute respiratory syndrome (SARS) coronavirus helicase ATPase and duplex-unwinding activities at micromolar concentrations.

**Acknowledgements:** This study was supported by Grant DO-02-39/2009 from the National Science Fund, Bulgarian Ministry of Education, Youth and Science.

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## **AP5. APPLICATION OF SILVER IN MEDICINE**

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## Session B. ROLE OF METALS IN NORMAL AND PATHOLOGICAL PROCESSES

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### BO1. CHANGES OF AORTIC WALL IN YOUNG SPONTANEOUSLY HYPERTENSIVE RATS UNDER DIET OF DIFFERENT SELENIUM CONTENT

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It is known that hypertension is associated with oxidative stress and endothelial dysfunction. Selenium (Se) containing cellular glutathione peroxidase (GPx-1) is the most abundant intracellular isoform of GPx antioxidant enzyme family. It plays a major role for neutralization of hydrogen peroxide molecules, formed into the cells. Hydrogen peroxide modulates the different functions of the endothelial cells: their growth and proliferation; apoptosis; endothelial-dependant vasodilatation; their barrier function; endothelial inflammatory answer; endothelial control of remodelling of the vessels. Tissue expression of selenoproteins is known to depend on daily Se intake. It is evaluated that diet containing 0,1 µg Se/g of food is enough for normal growth and reproduction. The aim of this study was to investigate the effects of different Se intake on the changes of aortic walls in young spontaneously hypertensive rats (SHR).

Twenty nine male 2-month old SHR (Okamoto-Aoki), divided into three groups, had received low (0,05µg Se/ g of food; n=10); adequate (0,11µg Se/ g of food; n=9) and high (0,25 µg Se/ g of food; n=10) selenium content diets during 8 weeks. Their systolic blood pressure (SBP) was measured indirectly using a tail cuff, before and after applied diets. A blood was taken under anesthesia of Pentobarbital Sodium (40 mg/kg i.p.). The serum Se concentration was determined, using flameless atomic absorption spectrophotometric analysis. The aortas of the rats, separated into 2 parts – thoracic and abdominal, were extirpated. The morphological changes were examined and described under light microscope, using longitudinal slices, stained by Hemalaun-Eosin. A measurement of the thickness of the aortic wall of each rat was performed at 10 points (including the thickest and the thinnest areas), using an ocular micrometer. Immunofluorescence cell staining was performed, using kits and protocols of "Santa Cruz Biotechnology, Inc.". Fluorescence microscopy image analysis was performed under laser scanning confocal microscope "Leica TCS

SPE". The F-test of Fisher (ANOVA), multiple range tests (LSD) and regression analysis, performed with statistical program SPSS, v. 15, were used for data analysis.

The serum Se concentration was significantly different between the groups ( $p < 0.05$ ). Fluorescence microscopy image analysis showed the different degree of fluorescent expression of GPx-1, depending on Se content of the blood. SHR with low Se had severe morphological changes of aortic walls in comparison to the other groups (endothelial lesions, subendothelial lipid and calcium deposits, proliferation of smooth muscle cells). Low Se diet caused significant thickening of thoracic ( $p = 0.008$ ) and abdominal ( $p = 0.004$ ) aortic walls. SBP of these rats was significantly increased ( $p = 0.004$ ), than the blood pressure of the rats from the other groups. Moderately strong inverse relationship between the thickness of aortic wall and the serum Se concentration was observed ( $r = -0.56$ ).

In conclusion, Se intake is a significant factor that affects status of aortic wall. GPx-1 deficit due to low Se intake may directly induce an increased oxidative stress level thus leading to endothelial dysfunction and aggravation of hypertension.

## **BP1. SELENIUM, HUMAN HEALTH AND SUPPLEMENTATIONS**

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Selenium (Se) is an essential trace element, with an atomic number 34, an atomic mass of 78.96 and a wide spectrum of biological effects of Se compounds. It has been recognized as an important antioxidant in the biosphere and in human health. It is a product of volcanic activity, found in rocks and other sources are biogenic materials like soil and water.

The effect of Se is strongly dependent on its chemical form (generally, organic compounds (selenomethionine, selenocysteine, and methylselenocysteine) of Se are more bioavailable than the inorganic forms (selenide, selenate, and selenite). Selenium is known to develop its biological activity via selenocysteine residue in the catalytically active centre of selenoproteins – they have their specific functions and all of them have an antioxidant function. Selenoproteins can be divided into two groups: group I, Se-Cys is located on the N-terminal position of the functional domain (glutathione peroxidases (GPx) - act as catalyst at hydrogen peroxides reduction by oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG); selenoprotein P- the major plasma selenoprotein, mainly produced in liver, lung and heart) and in group II, Se-Cys is presented in the C-terminal sequence (Thioredoxin reductase - redox protein that regulates several intracellular redox-dependent processes).

Diet is the major source of Se. The recommended dietary allowance (RDA) depends on the country, region, seasonal changes, age and sex of the individuals but for adults is about 55 µg. Meat, chicken, fish and eggs are protein-rich foods containing high levels of Se. Several studies have shown that Se bioavailability in meat is high because Se forms in foods of animal origin are mostly Se-Cys and Se-Met (Se-Met is an essential selenoaminoacid and is highly bioavailable). Fruit and fresh vegetables contain low concentrations of Se. This fact could be explained by the low protein fraction (and therefore, the high water content) of these products. Drinks and potable water are generally poor sources of Se. It has been found that Se concentrations in milk from different animal species decreases in the following order: human > sheep > goat > cow milk. It is known that Se concentrations in milk are negatively correlated with its fat content. Selenium's deficiency decreases selenoproteins expression and thus altering the biological processes mediated by them. Toxic concentrations and deficiency are very close to each other.

Nowadays there is a great interest into developing Se-enriched nutritional supplements, which are an alternative to increase its dietary intake for individuals in regions with very low environmental Se level. Selenium can be lost when food is processed or refined, due to its volatility. The possibility of fertilizing soil with Se salts has been suggested by adding sodium selenate to farmlands. Another alternative is supplementation of farm animals with Se: a) direct

application of Se to pastures to increase Se uptake by plants for animal feed; b) supply of sodium selenite or selenate incorporated into salt blocks or licks; c) direct administration of Se to animals by drenching with Se saltsolutions such as sodium selenite; and d) the use of Se pellets that slowly release Se in the animal's gut. The third strategy is human intake of multimicronutrient supplements containing Se: two different types can be distinguished: a) multi-vitamin and multi-mineral preparations containing inorganic Se, other trace elements and vitamins, and b) supplements based on *Saccharomyces cerevisiae* yeast. Se-enriched yeast supplements have been widely studied.

More researches are necessary in the supplementation field in order to have a better knowledge of the amount of Se biologically available for the human organism from Se supplements used nowadays.

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## BO2. METALLOENZYMES AND OXIDATIVE STRESS IN HEMODIALYSIS PATIENTS

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**Background:** There is a balance between antioxidant systems and generated reactive oxygen species (ROS) in the normal organism. When this balance is disturbed, an oxidative stress is developed, resulting in occurrence of different diseases. In hemodialysis (HD) patients oxidative stress is now well recognized. The reason for developing oxidative stress in HD patients, are the

high levels of urea, as a source of ROS. The condition of the patient might be further complicated when a diabetes mellitus is present. High blood sugar levels leads to higher oxidative stress levels, due to non-enzyme glycation of proteins and lipids. Hemodialysis itself, also contributes to formation ROS, due to dialysis membranes bio incompatibility.

**Aim:** The aim of this investigation is to study the changes of Superoxide dismutase (SOD), Catalase (CAT) and lipid peroxidation in dialysis patents, with and without diabetes.

**Materials and methods:** HD patients (n = 18), divided into two groups, were examined: diabetics (n = 10) and non-diabetics (n = 8). Immediately before and after dialysis venous blood was taken. To establish the antioxidant status, the activity of the enzymes SOD and CAT, and the levels of Malone dialdehyde (MDA) as a marker for lipid peroxidation, were measured.

**Results:** Compared to the control group (SOD  $2.52 \pm 0.22$  kU/gHb; CAT  $20.29 \pm 1.64$  kU/gHb), statistically significant differences in the levels of SOD and CAT in HD patients were reported, as the highest values showed diabetics before hemodialysis ( SOD  $3.25 \pm 0.68$  kU/gHb; CAT  $36.73 \pm 3.19$  kU/gHb). The levels of MDA showed a similar pattern: controls:  $1.73 \pm 0.07$   $\mu\text{mol/l}$ ; patients without diabetes  $2.94 \pm 0.57$   $\mu\text{mol/l}$ ; patients with diabetes  $3.66 \pm 0.19$   $\mu\text{mol/l}$ ;

**Conclusions:** The study reveals, that the HD patients (with and without diabetes) have SOD and CAT levels different than normal. Determining the activity of these enzymes may be a good indicator for monitoring oxidative stress in HD patients.

### BO3. EFFECT OF ZINC COMPOUND ON TRICHINELLOSIS IN MICE

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The present study investigated the effect of zinc compound ( $2\text{Gly.ZnCl}_2 \cdot 2\text{H}_2\text{O}$ ) on antioxidant system and parasite burden in mice experimentally infected with *Trichinella spiralis*. Cu/Zn-SOD activities (marker of antioxidant status), malonyl dialdehyde (MDA) concentration (index of endogenous lipid peroxidation), Zn liver and plasma concentrations, body weights, mortality and parasite burden were determinated at weeks 1<sup>nd</sup> and 8<sup>th</sup> post infection (pi).

Thirty – two BALB/c mice were allocated into three groups as follows: group 1, control (healthy and non-supplemented mice); group 2, experimentally infected with *T. spiralis* and non-supplemented, and group 3, experimentally infected with *T. spiralis* and supplemented with zinc compound. The mortality and body weights of the mice were recorded in each group. Mice from groups 2 and 3 were inoculated *per os* with 100 *T. spiralis* larvae. Treatment with Zn compound of groups 3 initiated 2 weeks before the infection and continued 3 weeks after the infection. Daily doses of 0.5 mg Zn/ 1 ml H<sub>2</sub>O in the form of  $2\text{Gly.ZnCl}_2 \cdot 2\text{H}_2\text{O}$  was administrated to each mouse from that group.

For biochemical assays, Cu/Zn-SOD activity in washed erythrocytes was determined according to Fridovich (1978). Lipid peroxidation was measured by serum MDA concentration as MDA-thiobarbituric acid adductor (Placer et al. 1966). Plasma and liver Zn contents were determined by atomic absorption spectrometry.

Following infection with *T. spiralis*, plasma Zn levels decreased and hepatic Zn levels increased significantly in the infected mice in comparison to controls. Zn supplementation increased Zn level in plasma and liver both in infected and control mice. Cu/Zn- SOD was not significantly changed in the infected mice in comparison with controls. Zn supplementation increased SOD –activity in the infected mice. The MDA levels in mice infected with *T. spiralis* were significantly higher than those in the control mice. However, the infected group receiving Zn had reduced lipid peroxidation. MDA content was lower in the infected and supplemented mice. Parasite burden was established at the 8th wpi. Zn supplementation reduced the parasites by 68%. There were no significant changes in the mortality of the infected mice due to Zn supplementation.



Zn supplementation increased body weight in the infected and control rats. Zn supplementation improved antioxidant defense system, body weight, and reduced muscle larvae but it did not affect mortality. Restored Zn status via Zn supplementation could improve antioxidant system in the infected mice. Zn supplementation may be of considerable benefit in improving antioxidant defense and overall health in mice infected with *T. spiralis*.

#### **BO4. СТРУКТУРЕН И ФУНКЦИОНАЛЕН АНАЛИЗ НА НОВ ТИП МЕТАЛОПРОТЕИН TS-РСТР ОТ ПАРАЗИТНИЯ НЕМАТОД *TRICHINELLA SPIRALIS***

**Г. Радославов**

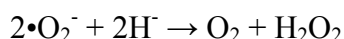
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#### **BO5. СУПЕРОКСИД ДИСМУТАЗА**

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Ензимът супероксид дисмутаза (СОД) представлява една от защитните антиоксидантни системи в организма. СОД са металоензими, които катализират дисмутацията на два супероксидни радикала до  $O_2$  и  $H_2O_2$ :



Молекулата на СОД е съставена от две събединици: апобелтък и метален кофактор, който може да бъде Cu, Zn, Fe, Mn или Ni. Активният ензим може да бъде открит в различни клетъчни органели и спомага за намаляване на уврежданията в клетъчните компоненти.

Според металния кофактор, участващ в изграждането на каталитичния център, във висшите растения са идентифицирани три изоензимни форми на СОД:

Cu, Zn- супероксид дисмутаза (най- широко разпространената форма при растенията, която е локализирана в цитозола, пластидите и пероксизомите; инактивира от водороден пероксид и калиев нитрат).

Mn- супероксид дисмутаза (локализирана в митохондриалния матрикс и в пероксизомите; не се инхибира нито от водороден пероксид, нито от калиев цианид);

Fe- супероксид дисмутаза (локализирана е в хлоропластите; инактивира се от водороден пероксид, но е устойчива по отношение на калиевия цианид).

Предполага се, че възникването на отделните изоформи в хода на еволюцията веоятно отразява различната достъпност на разтворимите преходни метални компоненти в биосферата, което на свой ред е свързано с нееднаквото кислородно съдържание през различните геологични етапи.

#### **BO6. IODINE FOR BODY USES AND DISEASE TRETMENT**

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## BP2. SOME CONSIDERATIONS ON THE FLUOROMETRIC ASSAY FOR HYDROXYL RADICAL AVERTING CAPACITY USING COBALT(II) – SALTS

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The determination of antioxidant capacity, in particular the choice of reliable method, is a serious problem (Tirzitis et al., 2010). Recently, there are many different methods, which explore various specific properties of the radicals determined, as well as of the antioxidants available. The fluorometric approach has been proposed and successfully applied in the last decade as a sensitive method, well known as HORAC (target is the hydroxyl radical) or ORAC (target is the peroxy radical). In the particular case of HORAC the determination is based on a Fenton-like reaction using cobalt as catalyst under physiological conditions. The suggested substance is CoF<sub>2</sub>. It is practically water-insoluble. Nevertheless, we observed satisfactory results even in the case when the catalyst was suspended in the medium. But the real concentration is not clear. In the original procedure described by Ou et al., 2001, picolinic acid was introduced as a stabilizer of CoF<sub>2</sub> in the reaction medium. On the other hand, one can not clearly cut between stabilization of the catalyst and eventual formation of Me(II)-chelator complexes, which is the way for inhibition of the reaction for generation of free radicals (OH•). The authors drive the attention exactly to that crucial point as a possible mechanism of action of many antioxidants. The picolinic acid also might be considered also as a possible synergistic agent in combination with the antioxidants, present in the reaction system. To avoid this ambiguity, we decided to apply another Co-salt, namely CoSO<sub>4</sub>·7H<sub>2</sub>O, which is water-soluble. In this way, the catalyst concentration is defined exactly and the observed course of the reaction is just normal. Different reaction conditions (reagent ratios) have been tested. In this sense, if we observe a change of the reaction course when it is performed according to the new conditions, the latter can be considered as a contribution of a possible antioxidant in the medium, acting as a Me(II)-chelator.

**Acknowledgements:** The present work was supported by the Interacademical Exchange Project “Interaction of collagen, platelets and phagocytes under physiological and pathological conditions (inflammatory processes, 2007-2010)” – IOCCP-BAS and IBP-Brno, AS CR, Czech Republic.

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### **BP3. SIGNIFICANCE AND CONTROL OF $\text{Na}^+$ IN THE ORGANISM OF ECONOMICALLY IMPORTANT ANIMALS**

**E. Arnaudova<sup>1</sup>, B. Georgiev<sup>2</sup>**

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Sodium is present in all organs and cell systems as biocatalyst. Its quantities are considerably higher in animals than in plant organisms. The most important  $\text{Na}^+$  role is to keep the water level in cell environment as well as homeostasis. It also maintains the asymmetric ion gradient ( $\text{Na}^+$  mainly outside and  $\text{K}^+$  mainly inside the cells) which is carried out by ATP. Because of its great mobility toward excretions two hormones take part in its regulation and contents in the organism. The aldosterone controls it quite economically not only at kidney level but in its excretion with the sweat, saliva etc as well. The vasopressin from the back part of pituitary gland limits the water emission and respectively but to a lesser extent that of  $\text{Na}^+$  by kidneys.

Control of an optimal and more stimulating effect of sodium on the economically important animals can be achieved by a gradual and slight augmentation of the fodder together with a free regime of water. We have found that in this way the aldosterone content decreases and that of vasopressin becomes higher. This makes animals tolerate the higher  $\text{Na}^+$  values and so the fodder utilization, development and fattening of animals (mainly pigs and poultry) could be optimized. But we must have in mind that a very high  $\text{Na}^+$  content in the forage accompanied by limited drinking water make it a dangerous poison.

### **BP4. USE OF HEMOPOIETIC BIOSTIMULATING AND FEEDING EFFECT OF IRON FROM NATURAL SOURCES**

**E. Arnaudova<sup>1</sup>, B. Georgiev<sup>2</sup>**

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Iron resorption and assimilation in the organism, especially the prophylactic ones, are highly limited both for iron inorganic and organic compounds. This is an important natural antitoxic protection. A good chance to assimilate iron offers the hem – its complex with hemoglobin. Data on this can be found in the remote past – the ancient Bulgarians mixed mare's milk and koumiss with horse blood. In the past and up to this day blood puddings from pig's blood are prepared in some countries. It would be most useful in such cases to use bird blood as its erythrocytes possess nucleus and hence DNA. In the bird slaughterhouses there is a lot of bird blood which is not used and runs out with the sewage. A system for its sterile collection could be created and the blood after a suitable preparation could be included as an addition to forages, even milks, cheeses, drinking mixtures, bread and others. This would be not only a prophylactic action but would have a strong feeding and economic effect in poultry-farming and in other directions too, especially on the background of the common protein deficiency. We offer here a plan for such project.

## Session C. **METAL TOXICITY AND CARCINOGENICITY. METALS AND ENVIRONMENT**

### **Chairpersons:**

**Assoc. Prof. Olga Baicheva, PhD**

*Institute of Experimental Morphology, Pathology and Anthropology with Museum,  
Bulgarian Academy of Sciences*

**Assoc. Prof. Anna Damianova, MD, PhD**

*Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences*

### **Secretary:**

**Lora Dyakova**

*Institute of Neurobiology, Bulgarian Academy of Sciences*

### **CO1. INFLUENCE OF STEROID GLYCOSIDES ON MINERAL COMPOSITION OF THE PLANTS INFECTED BY ROOT-KNOT NEMATODE, *MELOIDOGYNE ARENARIA***

**O. Baicheva<sup>1</sup>, A. Damianova<sup>2</sup>, S.V. Zinovieva<sup>3</sup>, Zh.V. Udalova<sup>3</sup>, I. Vladov<sup>1</sup>, I.S. Vasilieva<sup>4</sup>,  
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In work data about influence of furostanol glycosides on the maintenance of elements in tissues of tomatoes invasion by root-knot nematode are presented. It is shown, that processing of plants allows stabilizing the mineral composition broken by action of nematode.

### **CO2. INVESTIGATIONS OF COMPLEX TREATMENT BY ADAPTOGEN AND GAMMA – IRRADIATION ON THE MINERAL COMPOSITION OF INFECTED WITH *MELOIDOGYNE ARENARIA* TOMATO PLANTS**

**A. Damianova<sup>1</sup>, I. Vladov<sup>2</sup>, O. Baicheva<sup>2</sup>, S.V. Zinovieva<sup>3</sup>, Zh.V. Udalova<sup>3</sup>**

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Studies on the effect of gamma-irradiation and treatment with adaptogen on the micro-element content of infected with *Meloidogyne arenaria* tomato plants were carried out.

Adaptogen (furostanol glycoside) has been extracted from cell culture of *Dioscorea deltoidea*. The experiments were made under laboratory conditions ( T-20-23°C). The plants (*Tiny Tim*) were cultivated on soil, sterilized by heating. Tomato seeds were treated with 0.1 % solution of adaptogen.

By soaking for 1 hour and irradiated with different doses of gamma-rays. The amount of Zn, Cu, Fe, Mn and Mg were analyzed by atomic absorption. A positive effect of gamma - rays and adaptogen on the microelement content of the plants was observed.

### CO3. CHEMICAL SPECIATION IN NATURAL AND BRINE SEA WATERS

**S. Tepavitcharova<sup>1</sup>, T. Todorov<sup>1</sup>, D. Rabadjieva<sup>1</sup>, M. Dassenakis<sup>2</sup>, V. Paraskevopoulou<sup>2</sup>**

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A combined approach consisting of monitoring and thermodynamic modeling was used in order to calculate the concentration of trace element species in water samples of a broad salinity range and to explain their chemical behaviour. The study was performed on water samples (fresh, marine, hyper-saline) taken from the area of Burgas Bay.

The ion association model based on Debye-Hückel theory using the *sst2008.dat* database and the ion interaction model based on Pitzer theory using a new *pit2010.dat* database were compared and combined for the purposes of this study. The new *pit2010.dat* database combines the *sst2008.dat* database and the *pitzer.dat* database of the PHREEQCI computer program as well as the thermodynamic data for the elements Fe, Mn, Cu, Zn, Cd and Pb and their Pitzer ion interaction parameters.

The results showed that (i) the predominant species in fresh waters were free ions of  $\text{Mn}^{2+}$  (73.6%),  $\text{Zn}^{2+}$  (58.0%) and  $\text{Cd}^{2+}$  ions (78.3%) as well as carbonate species  $\text{CuCO}_3^0$  (81.8%),  $\text{PbCO}_3^0$  (77.2%) and hydroxy species  $\text{Fe}(\text{OH})_3^0$  (55.2%) and  $\text{Fe}(\text{OH})_2^+$  (35.6%); (ii) an increase in chloride species  $\text{MnCl}_n^{2-n}$  (n = 1-4) for Mn, Zn, Cu, Pb and Cd, and of the hydroxy species  $\text{Fe}(\text{OH})_2^+$  for Fe was calculated for sea- and hyper-saline water.

### CO4. HEAVY METALS CONTENT IN SOIL NEAR NON-FEROUS METALS PRODUCTION FACILITY AND DOMESTIC WASTES LANDFILL IN THE AREA OF KARDZHALI TOWN

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#### Abstract

The predominant soil types in the region of Kardzhali are maroon forest soils - highly leached and poorly podzolized. Most of the soils have medium to high erosion ability - II-III

degree, and the pH is neutral to slightly alkaline (7-7.8). Few are weak and moderate acidic pH (6.7-5). Soils are mostly shallow to moderately deep, poor in nutrients.

In the region of Kardzhali main pollutants are heavy metals from mining-processing and metallurgical business activities of “Gorubso-Kardzhali” - AD and LZC - AD, Kardzhali.

In the paper are presented results of soil contamination mainly with lead, cadmium, copper and zinc.

### **Увод**

Преобладаващият почвен тип в Област Кърджали са канелените горски почви – силно излужени и слабо оподзолени. Срещат се също в по-малък размер кафяви горски почви, рендзини (хумосно-карбонатни), алувиални, канелено-подзолисти, делувиялни и в незначителен размер други почвени типове. Най-плодородните почви са по долините на р. Арда и нейните притоци и в северните части на областта на границата с Хасковска област.

По-голяма част от почвите имат средна до висока степен на ерозийност – II-III степен, а pH е неутрално до слабо алкално (7-7.8). Малка част са със слабо и средно кисела реакция pH (6.7-5). Почвите са предимно плитки до средно дълбоки, бедни на хранителни вещества. Обработваемите земеделски земи са разпокъсани и малки по площ. Характерна особеност в този район е, че обработваемата площ не образува компактни масиви, както е в другите райони на страната, а е разчленена на голям брой дялове, различни по размер, форма и положение [1].

В района на гр. Кърджали основни замърсители са тежките метали от минно преработвателната и металургична дейност на предприятията “Горубсо-Кърджали”-АД и ОЦК-АД, Кърджали [2].

В настоящата статия се представят резултати от замърсяването на почвата основно с олово, кадмий, мед и цинк.

### **Материал и методи**

Пробонабирането на почвени проби е изпълнено в съответствие с изискванията на ISO 10381 – 1 и БДС 17.4.5.01-85 за анализ на тежки метали. В зависимост от приетите в хигиенната практика критерии са определени представителни пробовземни пунктове. Броят им е 21 и са разположени в близост до източниците на замърсяване, до натоварени с автомобилен транспорт кръстовища, отдалечени от замърсителите и в близост до детски градини и училища.

Почвените проби са взети според “Метода на диагоналите“, с условно разделяне на територията за пробовземане на парцели от 0,5 ha, с анализ на 5 проби от всеки парцел. Единичните проби от всяка пробоземна точка са смесени на място с оформяне на средна проба от 1 kg. Средните проби са съставени от 5 единични проби, взети равномерно от площта на стационарната площадка. Пробовземането е извършено от дълбочина 0-10 cm.

Определянето на съдържанието на токсични елементи в минерализатите е извършено чрез методите на атомно-абсорбционната спектроскопия чрез пламъкова ААС, съгласно ISO 11047:1998, след съответна подготовка на пробите – минерализация по БДС 17.4.4.01-79 [3].

Подготовката и аналитичното определяне на изследваните елементи е извършено според стандартизираните методи (Табл. 1).

**Таблица 1. Използвани лабораторни методи за анализ на тежки метали**

Елемент	Метод за анализ	Аналитична техника	Граница на откриване, mg/kg
Кадмий	БДС 17.4.4.04-80, ISO 11047	FAAS	0,25
Мед	БДС 17.4.4.03-80, ISO 11047	FAAS	0,30
Цинк	БДС 17.4.4.05-80, ISO 11047	FAAS	0,32
Олово	БДС 17.4.4.02-80, ISO 11047	FAAS-2	2,5

## Резултати и обсъждане

Основните индустриални замърсители на почвите в района на град Кърджали са: Оловно-цинковият комплекс (ОЦК АД), "S&B Industrials Minerals" АД (бивш „Бентонит” АД) и Хвостохранилището на „Горубсо-Кърджали” АД. Тежките метали попадат в почвата от емитираните във въздуха газове от посочените промишлени предприятия.

Антропогенното замърсяване от производствената дейност зависи и от посоката на преобладаващите ветрове, които за района са северно и северозападно от града.

Освен посоката на преобладаващите ветрове и на фоновото ниво, почвената киселинност е от голямо значение, тъй като от нея зависят ПДК на тежките метали в почвите (Наредба № 3/01.08.2008 год. за нормите за допустимо съдържание на вредни вещества в почвите).

Почвите в района на гр. Кърджали са карбонатни черноземни-смолници, средно мощни и леко глинести. Съставът и почвената реакция са фактори за сравнително активната миграция на тежките метали по вертикалния профил на почвата.

В **таблица №2** са представени данни за съдържанието на тежки метали (кадмий, мед, цинк и олово) в почви, осреднени за периода 2008-2010 г.

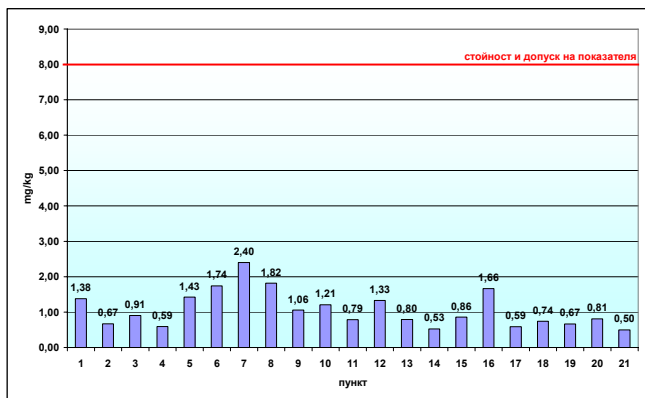
**Таблица 2. Съдържание на тежки метали, осреднено за периода 2008-2010 г. в (mg/kg)**

№	Пункт	Съдържание на тежки метали, mg/kg			
		Cd	Cu	Zn	Pb
1	гр. Кърджали - кв. Байкал, ул. "Ген. Вл. Стойчев" №2	1.38	9.87	127.85	222.56
2	гр. Кърджали - на изхода за гр. Хасково	0.67	11.64	122.59	114.65
3	гр. Кърджали, бул. "Беломорски" - двора на ЦДГ "М. Горкий"	0.91	25.78	198.25	289.50
4	гр. Кърджали, кв. "Студен кладенец", ул. "Веслец"	0.59	42.09	99.58	31.11
5	гр. Кърджали, кв. "Студен кладенец" – ОПУ	1.43	41.64	200.12	405.31
6	гр. Кърджали, ЖП Гара, бл 81	1.74	22.89	205.23	441.28
7	гр. Кърджали, ПГ "Васил Левски" - двора	2.40	27.68	387.28	688.67
8	гр. Кърджали, ДМСГ	1.82	22.28	115.20	221.03
9	гр. Кърджали, кв. "Студен кладенец", ул. "Доспат" №1 А	1.06	182.66	103.38	239.54
10	гр. Кърджали, кв. "Ст.кладенец", СОУ "Св.Кл. Охридски"	1.21	19.51	113.82	293.95
11	гр. Кърджали, ЦДГ "Вяра, Надежда, Любов"	0.79	17.79	116.72	49.58
12	гр. Кърджали, кв. Възрожденци", СОУ "П.Р. Славейков"	1.33	17.36	95.73	132.36
13	гр.Кърджали, кв. "Веселчани", ул. "Победа" №9	0.80	21.05	115.42	38.67
14	гр. Кърджали, кв. "Гледка", ул. "Тина Киркова" № 4	0.53	21.08	99.64	27.08
15	гр. Кърджали, кв. "Горна Гледка", ул. "К.Ляtifова" №17	0.86	15.33	78.85	35.94
16	с. Островица, общ.Кърджали	1.66	24.98	164.27	64.27
17	гр. Кърджали – изхода (пътя за гр. Ардино)	0.59	10.15	78.06	22.88
18	гр. Кърджали кв. "Прилепци" – срещу ПС за питейна вода	0.74	12.97	51.33	21.61
19	с. Вишеград, общ. Кърджали	0.67	23.98	54.30	25.83
20	с. Глухар, общ. Кърджали	0.81	13.98	193.07	36.15
21	с.Енчец, общ.Кърджали	0.50	16.13	134.88	375.00
Средна обща стойност		1.35	1.07	28.61	135.98

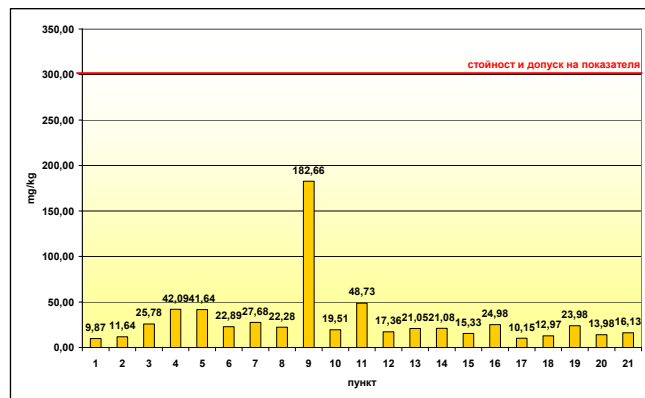
От изложените данни в Табл. 2 и Фиг.1-4 се констатира, че съдържанието на кадмий в почвите на изследвания район не надвишава ПДК, съгласно Наредба 3/2008 г. [4]. Най-високата концентрация на кадмий (2.40 mg/kg) е отчетена в пункт № 7 - Професионална гимназия "Васил Левски", която се намира в близост до фабриката за добив и преработка на оловно-цинкови руди - „Горубсо-Кърджали” – АД.

Съдържанието на мед и цинк е под ПДК във всички пунктове. Максимални стойности на мед са определени в пункт № 9 - кв. "Студен кладенец", ул. "Доспат" №1А (182,66 mg/kg), а на цинк (387.27 mg/kg) отново в пункт № 7 - ПГ "Васил Левски".

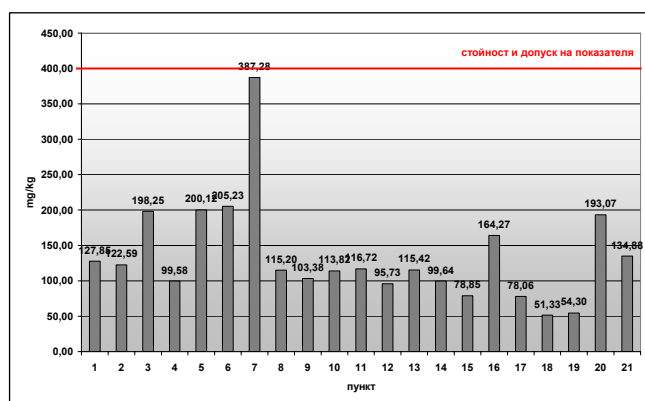
Приблизително в 50% от пунктовете съдържанието на олово е над нормата за максимално допустима концентрация (200 mg/kg) от 1.10 до 3.44 пъти. Най-масивното замърсяване с олово от изследваните проби почви е в района на ПГ“Васил Левски”. Високи средни стойности за периода са отчетени и в пункт - ЖП гара. Вероятното натрупване на олово е не само от ОЦК-АД, а и от Хвостохранилището на „Горубсо-Кърджали”-АД, от транспортирането на оловен концентрат в близост до този пункт и автомобилния трафик от прилежащия булевард. Общата средна концентрация на олово за 21-те пункта не надвишава ПДК.



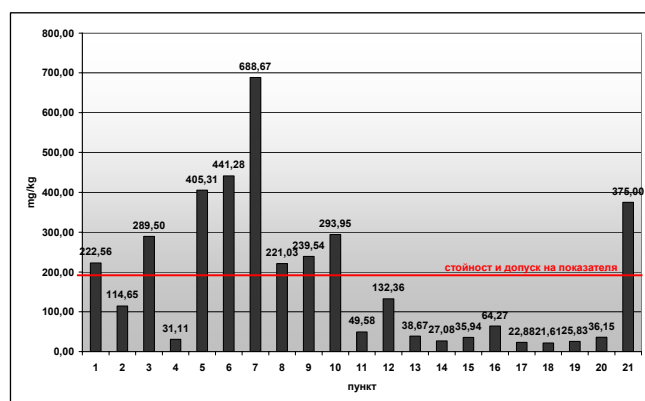
Фиг. 1 Съдържание на кадмий в почви – за периода 2008-2010 г.



Фиг. 2 Съдържание на мед в почви – средно за периода 2008-2010 г.



Фиг. 3 Съдържание на цинк в почви – средно за периода 2008-2010 г.



Фиг. 4 Съдържание на олово в почви - средно за периода 2008-2010г.

Сравнявайки данните за съдържание на тежките метали (кадмий, мед, цинк и олово) в почвата на района на гр. Кърджали за периода 2008-2010 г., се наблюдава значително по-високата им концентрация в края на проучването, което потвърждава кумулативните и консервативни свойства на почвата като среда. Резултатите са представени в таблица № 3.

**Таблица 3. Средни стойности, медиани и стандартни отклонения на тежки метали в почви за периода 2008-2010г.**

Статистически показател	Cd			Cu			Zn			Pb		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Средна стойност	0.500	0.465	1.516	44.150	7.102	17.143	168.600	42.143	201.357	36.950	19.853	70.626
Медиана	0.400	0.280	1.250	39.500	0.870	14.000	173.000	16.880	180.000	38.000	2.750	48.000
Стандартно отклонение	0.138	0.631	0.868	20.559	15.533	9.873	32.107	60.520	133.312	5.889	42.232	52.256



## Изводи

Почвата, замърсена вторично от атмосферата и депо за тежките метали, превишаващи регламентираните норми, би могла да бъде потенциален източник за замърсяване на подпочвените води, растителните и животинските продукти с локален произход.

За разглеждания период (2008-2010 г.) се установява, че приблизително в 50% от пунктовете съдържанието на олово в почви е над нормата за максимално допустима концентрация (200 mg/kg) от 1.10 до 3.44 пъти. Съдържанието на кадмий, мед и цинк е под ПДК.

На регионално ниво, институциите с отговорности към управлението на качеството на околната среда и общественото здраве в региона е необходимо да подхождат единно за успешното постигане на ефективни решения на въпросите относно:

- аерозолното замърсяване от Хвостохранилище „Горубсо - Кърджали” АД;
- оптимизиране пречиствателните съоръжения на ОЦК АД и S&B Industrials Minerals АД в съответните производства;
- популяризиране сред частните стопани отглеждането на култури с малък потенциал за натрупване на тежки метали и подходящо залесяване.

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## CO5. HEALTH STATUS OF THE STUDENTS IN KARDZHALI REGION

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### Abstract

In this article is presented a study of the health status of students in Kardzhali Region for the period 2008-2010. There were used a RHC Kardzhali's statistical databases and the data of prophylactic medical examinations of students from 7 to 18 years old.

Analysis of the individual assessment of height and body mass of Kardzhali Region students in this age group shows that 85% are into the norm on characteristics height and body mass.

Leading in the structure of morbidity are: obesity, visual disturbances, chronic diseases of tonsils and adenoids, spinal deformities, asthma, chronic bronchitis, etc.

The increase of detected diseases in prophylactic examinations of the students is statistically significant, i.e. may refer to the entire population of children, not just the total number of students examined in the sample ( $P < 0.0001$ ).

### Увод

Общественото здраве се характеризира с изключителна сложност, многоплановост и многомерност. Неговото изучаване като цялостен системен феномен изисква да се използва широка гама от индикатори и показатели, отнасящи се за всички групи здравни явления.

Засега в практиката са се наложили главно три групи индикатори за анализ на общественото здраве: демографски показатели, заболяемост и физическо развитие [1].

Целта на настоящото проучване е да се направи анализ на здравословното състояние на ученици във възрастова група 7-18 години от област Кърджали за периода 2008-2010 година.

Информация за физическото развитие и здравословното състояние на учениците се предоставя от медицинските специалисти от учебните заведения на област Кърджали и се анализира в Регионална инспекция за опазване и контрол на общественото здраве (РИОКОЗ).

Област Кърджали е една от първите, организирани системата за ученическо и детско здравеопазване. Към 30.06.2010г. в този сектор от здравеопазването работят 132-ма медицински специалисти, назначени по Наредба №3/24.04.2000г., които обхващат 81 учебни и 74 детски заведения. В малките села се практикува обединяването на няколко детски заведения, обслужвани от един здравен работник.

### Материал и методи

Първичната информация е събрана от статистическите масиви на РЦЗ – Кърджали и данните от профилкатичните медицински прегледи на ученици от 7 до 18 години, извършени през периода 2008 – 2010 г. Извършен е честотен и графичен анализ, като за сравняване на заболяемостта е използван точният критерий на Фишер.

### Резултати и обсъждане

Общият брой на учениците, обслужвани от звено “Училищно и детско здравеопазване” в област Кърджали, е 17 437. През учебната 2009/2010 година профилактичен преглед са преминали 16 591 (95.15%) ученици. В сравнение с предходната година, когато прегледаните от личните лекари ученици са 17 332 (96.85%), през настоящата те са с 1.7% по-малко.

Физическото развитие на учениците се определя по антропометричните показатели (ръст и телесна маса), които се сравняват с нормите за съответната възраст и пол.

При индивидуалната оценка на физическото развитие на учениците се оформят три групи: норма, разширена норма и извън нормата.

Броят на учениците, на които е измерен ръста, е общо 17 437, т.е. всички, които подлежат на антропометрични измервания. С най-голям относителен дял са учениците в норма (средно 85.9%) при двата пола и в двете възрастови групи. Във втора група (разширена норма) са 11.4% от общия брой ученици. Извън нормата с много нисък ръст са 1.7% момчета и момичета. С много висок ръст са 1.1% от учениците (Табл.1).

**Таблица 1. Динамика на ръста при учениците за периода 2008 - 2010 година**

Година	I гр. норма				II гр.разширена норма			
	Брой ученици / $\pm 1S$ /				Брой ученици между / $\pm 1S$ / и / $\pm 2S$ /			
	7-14 г.		14-18 г.		7-14 г.		14-18 г.	
	момчета	момичета	момчета	момичета	момчета	момичета	момчета	момичета
2008	88.7	88.1	92.2	91.4	9.2	9.2	6.7	6.3
2009	87.1	87.0	87.7	90.5	10.3	10.3	10.1	7.7
2010	84.5	84.6	87.0	87.4	12.4	12.5	10.2	10.4

Година	III гр. извън нормата							
	под / $x - 2S$ /				над / $x + 2S$ /			
	7-14 г.		14-18 г.		7-14 г.		14-18 г.	
	момчета	момичета	момчета	момичета	момчета	момичета	момчета	момичета
2008	1.2	1.5	0.7	1.1	0.9	1.2	0.4	1.2
2009	1.5	1.6	1.1	1.0	1.1	1.1	1.1	0.8
2010	1.9	1.9	1.4	1.4	1.1	1.1	1.4	0.8

За тригодишен период се наблюдава тенденция към понижаване на процента ученици с нормален ръст средно с 4.2%. Относителният дял на учениците във втора група (разширена норма) е с 3.6% повече спрямо 2008 г., а в трета група (извън нормата с много нисък ръст) - с 0.6 %. Тенденцията при ученици с много висок ръст се запазва на относително постоянно ниво и през трите години.

Броят на учениците, на които е измерена телесна маса, е общо 17 437 (100.0%). В норма са 83.3% от тях, а в разширена норма 12.5%. Извън нормата с много ниско тегло са 1.5% от общия брой. С много голямо тегло са средно 2.7% (Табл. 2).

**Таблица 2. Динамика на телесната маса при учениците  
за периода 2008 - 2010 година**

Година	I гр. норма				II гр.разширена норма			
	Брой ученици / $x \pm 1S$ /				Брой ученици между / $x \pm 1S$ / и / $x \pm 2S$ /			
	7-14 г.		14-18 г.		7-14 г.		14-18 г.	
	момчета	момичета	момчета	момичета	момчета	момичета	момчета	момичета
2008	86.2	86.2	88.1	89.6	9.5	9.2	9.3	7.1
2009	82.6	81.0	85.2	87.5	11.4	13.2	11.0	9.0
2010	81.3	81.4	84.2	86.4	13.6	14.0	11.3	11.1

Година	III гр. извън нормата							
	под / $x - 2S$ /				над / $x + 2S$ /			
	7-14 г.		14-18 г.		7-14 г.		14-18 г.	
	момчета	момичета	момчета	момичета	момчета	момичета	момчета	момичета
2008	2.1	2.2	1.3	1.6	2.2	2.4	1.3	2.1
2009	1.8	2.6	1.4	1.3	4.2	3.2	2.4	1.8
2010	1.7	1.4	1.7	1.3	3.4	3.2	2.8	1.7

За тригодишен период се наблюдава запазване тенденцията за понижаване на нивата на телесна маса в първа група (норма) и трета група (извън нормата под ( $x-2S$ )) и повишаване във втора група (разширена норма) и трета група (извън нормата над ( $x+2S$ )).

Резултатите от измерванията показват, че е намалял относителният дял на учениците, които са с ръст и телесна маса в норма. Наблюдава се положителна тенденция по двата показателя във втора група разширена норма. Процентът на учениците, които изостават във физическото си развитие по показател "ръст" се увеличава, а по показател "телесна маса" - намалява. Увеличава се и делът на учениците, които са с ръст над две стандартни отклонения, а с телесна маса в тази група - намалява.

### **Изследване на физическата дееспособност**

Едно от задълженията на медицинския специалист според Наредба № 3/2000 г. на МЗ е определяне на индивидуалната оценка за физическата дееспособност на учениците.

След обобщаване на подадената от тях информация се отчита, че 17 365 ученика са покрили тестовете за физическа дееспособност.

Промяната на брой ученици получили оценка над среден (3) при изследване на физическата дееспособност (60.7%; 59.0% и 60.8%) е статистически значима ( $P < 0.0001$ ).

Промяната на брой ученици освободени от часовете по физическо възпитание (50.0%; 36.3% и 40.3%) не е статистически значима ( $P = 0.134$ ).

Броят на учениците, включени в групи по лечебна физкултура за 2010 г. е 53 срещу 68 за 2009 г. и 52 за 2008 г. Колебанието на брой ученици, включени в групи по лечебна физкултура в училище (36.5%; 14.7% и 15.1%), е статистически значимо ( $P = 0.009$ ).

### **Оценка на основните профилактични прегледи и диспансерно наблюдение на учениците**

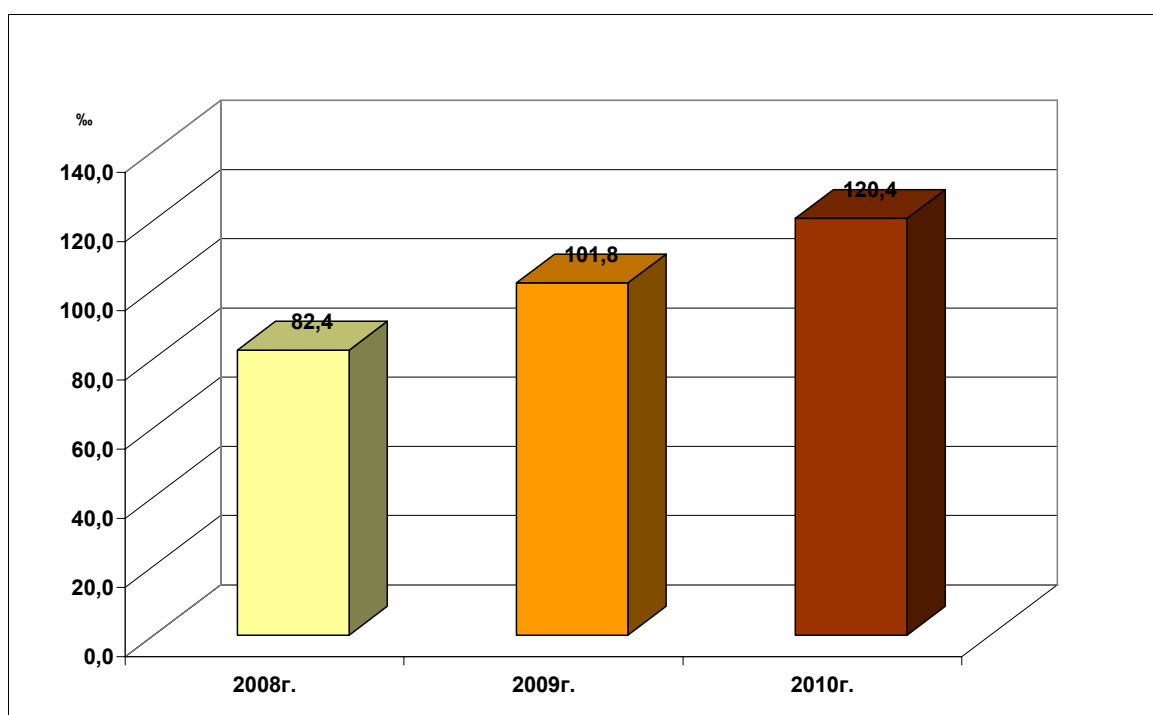
Съгласно Наредба №39/2004 г. "За профилактичните прегледи и диспансеризацията" [2] през 2010 година са прегледани общо 16 591 ученици от област Кърджали. Това

представлява 95.15% от общия им брой. През предходната година относителният дял на прегледаните ученици е бил 96.42%, а през 2008 г. – 96.25%.

Част от учащите се посещават ОПЛ по родните си места и не предоставят данни от извършените профилактични прегледи. За уточняване на диагнозата се налага необходимостта от преглед и консултация в специализирани болнични заведения. Тези обстоятелства, свързани с липсата на материална възможност в голям брой семейства, са причина броят на установените диагнози сред учениците от област Кърджали да е твърде малък - 1 998 (12.04%) от всички, обхванати с профилактични прегледи.

С профилактични прегледи за учебната 2009/2010 г. са обхванати 16 591 ученика и са регистрирани 1 998 заболявания, т.е. 120 от 1 000 прегледани са с отклонение от здравословното състояние. Наблюдава се тенденция към нарастване на общия брой открити заболявания. За 2008 г. честотата на случаите на 1 000 прегледани ученика е 82.4 ‰, за 2009 г. тя е 101.8 ‰, като през 2010 г. достига 120.4‰ (Фиг. 1).

Нарастването на откритите заболявания е статистически значимо, т.е. може да се отнесе към цялата популация от деца, а не само за общия брой прегледани ученици в извадката ( $P < 0.0001$ ).



Фиг.1 Динамика на откритите заболявания при основните профилактични прегледи на учениците от област Кърджали

### Оценка на основните профилактични прегледи

Анализът на резултатите от профилактичните прегледи показва, че през 2010 г. най-голям е броят на учениците със затлъстяване - 611 (36.8 ‰), а в предходната година те са – 31.6 ‰. Увеличението е с 5.2 ‰. Промяната на затлъстяването е статистически значима ( $P < 0.0001$ ).

На второ място в структурата на моментната болестност са смущения в зрението - 284 (17.1‰). През последните три години процентът на учениците с този проблем се увеличава с 3.5‰. Промяната на смущения в зрението (1.4%; 1.4% и 1.7%) е статистически значима ( $P = 0.008$ ).

На трето място в структурата на моментната болестност са учениците с хронични заболявания на тонзилите и аденоидните вегетации - 100 (6.0‰), което е увеличение с 3.9‰ в сравнение с последните две години. Промяната е статистически значима ( $P = 0.004$ ).

Следват гръбначните изкривявания, като са регистрирани 76 случая, т.е. 4.6‰. През предходната година броят им е бил приблизително 2 пъти по-нисък.

Други заболявания, които бележат висок ръст през 2010 г., са: астма (4.5‰), хроничен бронхит (4.2‰), олигофрения (4.0‰), епилепсия (3.7‰), пневмония (3.6‰) и т.н. От тях със статистическа достоверност са хроничен бронхит ( $P = 0.035$ ) и олигофрения ( $P < 0.0001$ ).

### **Диспансерно наблюдение на учениците от област Кърджали през 2010 г.**

Съгласно Наредба №39 на МЗ от 2004 г. диспансеризацията се осъществява от лекари специалисти от лечебните заведения. В здравно профилактичните карти на учениците личните лекари отразяват диагнозите, за които те се диспансеризират. За 2010 г. от прегледаните 16 591 ученика на диспансерен отчет се водят 1 151, което представлява 6.94% от прегледаните ученици и 57.60% от откритите заболявания. В сравнение с предходната 2009 г., когато на диспансерен отчет са били 1095 ученика (6.32%), през настоящата отчетност прегледаните ученици са с 0.62% повече.

От заболяванията, подлежащи на диспансерно наблюдение, най-голям дял от общия брой на откритите заболявания имат: затлъстяване (30.58%), гръбначни изкривявания (3.80%), бронхиална астма (3.70%), хроничен бронхит (3.50%), олигофрения (3.30%), епилепсия (3.05 %), вродени аномалии (1.90%), гастрит и дуоденит (1.60%), вродени аномалии на сърдечно-съдовата система (1.30%), хипертонична болест (1.00%) и т.н.

На територията на областта в гр. Ардино се намира Професионална гимназия, в която се приемат деца предимно със заболявания на дихателната система. Общият брой на диспансеризираните ученици с хроничен бронхит и астма е свързан с контингента на това учебно заведение. Високият процент олигофрения и епилепсия аналогично може да бъде свързан с контингента на Помощно училище с интернат “Д-р Петър Берон” в Кърджали, където се обучават предимно деца с интелектуален дефицит.

### **Изводи**

Анализът на физическото развитие на учениците във възрастова група 7-18 години показва, че 85% от тях са в норма по показатели ръст и телесна маса.

При тестовете за физическа дееспособност 100% от учениците са покрили нормите за съответната възраст. Състоянието на 4.1% от учениците, страдащи от хронични заболявания, не им позволява да участват в часовете по физическо възпитание.

Водещи в структурата на заболяемостта са: затлъстяване, смущения в зрението, хронични заболявания на тонзилите и аденоидните вегетации, гръбначни изкривявания, астма, хроничен бронхит и т.н.

Нарастването на откритите заболявания при профилактичните прегледи на учениците е статистически значимо, т.е. може да се отнесе към цялата популация от деца, а не само за общия брой прегледани ученици в извадката ( $P < 0.0001$ ). Тези данни са основание за стартиране на детската компонента на програма СИНДИ, чиято стратегическа цел е подобряване на здравето на децата и намаляване на риска от преждевременно развитие на хронични неинфекциозни болести.

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# CP1. PRELIMINARY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY STUDY ON PRESENCE OF HEAVY METALS IN UNCULTIVATED SOIL AREAS OF STARA ZAGORA AND CHIRPAN MUNICIPALITIES

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## Background

Under the conditions of anomalously high amount of heavy metal arriving with atmospheric precipitation, an overwhelming of heavy metals is retained mainly in the upper layers of soil. The presence of heavy metal causes changes in the status of humic substances, structure, pH and biological properties of the soil, which results in partial (or complete in the some cases) loss of the soil fertility (1).

The fact that last 5 years the air of the Stara Zagora Region at regular intervals was polluted, pursued us to study presence of paramagnetic ions of heavy metals in soil samples collected from that region using EPR spectroscopic method.

## Materials and methods

### *Soil Samples*

A number of 7 double soil samples from Municipality of Stara Zagora and 4 double soil samples from Municipality of Chirpan were collected. Each soil sample was taken twice: first from the surface layer and second from the depth of 20 cm.

### *Electron paramagnetic resonance (EPR) study*

All EPR measurements were performed at room temperature on a X-band EMX<sup>micro</sup>, spectrometer Bruker, Germany, equipped with standard Resonator. All EPR experiments were carried out in triplicate.

## Results and Discussion

The g values for each EPR signal registered in the EPR spectra of the studied samples were calculated using the spectral processings WIN-EPR and Simphonia softwares. Based on the other authors results and calculated g values the following metal ions were found: values of 2.8825 and 3.6115 we related to the presence of  $\text{Mn}^{4+}$  (3). A well-resolved signal with a hyperfine structure of six lines centered at  $g=2.16$  was unambiguously attributed to isolated  $\text{Mn}^{2+}$  ions (4). A signal with a weak intensity centered at  $g=4.24$  is characteristic for the isolated  $\text{Fe}^{3+}$  ions (4). Moreover, signals centered at  $g=4.13$  (weak) and  $g=2.056$  (strong) were also registered and they might be related to presence of  $\text{Fe}^{3+}$  ions in tetrahedral and octahedral coordinations (1). In some of the spectra a strong EPR signal at  $g=2.00$  might be attributed to presence of semiquinone free radicals. A broad EPR signal at  $g=2.00$  with average width of 1000 G was also registered and attributed to  $\text{Cr}^{3+}$  presence (5). In some samples signals at  $g=2.30$  might be due to the presence of  $\text{Cu}^{2+}$  (2).

## Conclusion

Since, in most of the studied samples was recorded signal of  $\text{Cr}^{3+}$  ions might be assumed that their presence was largely mask for the presence of other radicals and/or paramagnetic species i.e. presence of other heavy metal ions in the tested soil samples.

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## **CP2. BRASSICACEAE (CRUCIFERAE) SPECIES - SUITABLE PLANTS FOR SOIL PHITOREMEDIATION**

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Phytoremediation of metals is a cost-effective "green" technology based on the use of specially selected metal-accumulating plants to remove toxic metals from soils. Specifically, several subsets of metal phytoremediation exist including phytoextraction. The initial scientific efforts in this area focused on the selection of the best plants with natural ability of metals hyperaccumulation. However, since metals may be bound too tightly to soil components, genetic potential to accumulate metals does not always translate into effective phytoextraction. The unique plants that survive in serpentine soils have been used in the process of phytoremediation. These plants are the subject of many studies to improve their resistance to high concentrations of heavy metals in serpentines and increase hiperacomulation. There are more and more wild and cultivated species, which help in different regions of the Earth and account for the exploiter's needs (Garg et al., 2009).

For now are known about 400 plants that hyperaccumulate metals. Most of them are members of the families *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Cyperaceae*, *Cunouniaceae*, *Fabaceae*, *Flacourtiaceae*, *Lamiaceae*, *Poaceae*, *Violaceae*, and *Euphobiaceae*. *Brassicaceae* had the largest number of taxa 11 genera and 87 species (Prasad and Frietas, 2003, Palmer et al., 2001).

The present study aimed to find a potential species from *Brassicaceae* for use in cleaning up the soil in industrial polluted regions. For this purpose, we compared the data for these plants and used as hyperaccumulators and phytoextractors in world experience with the data for such species in Bulgaria (Pavlova, 2004, Simeonov and Simeonova, 2006). Representatives of the genera *Brassica*, *Thlaspi* and *Alyssum* were rated as promising. The results of this research showed that from crops radish and mustard plants of family *Brassicaceae* are hyper accumulator plants that can concentrate heavy metals in their different parts, thus they can be used for remediation of polluted area.

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### **CP3. THE ACTION OF METAL CONTAINING PESTICIDES ON THE LIVING ORGANISMS AND HUMAN HEALTH**

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In the present investigation the authors discussed and analysed the most important problems concerning the biological and health effects of the pesticides.

The pesticides are chemicals of different classes with different structure and chemical, physical and biological properties. The term “pesticide” originated from two Latin words – “pest” and “cido”. “Pest” is an organism, which causes destruction, disease on plants and animals, while “cido” means destroy or kill [1].

Subclasses of pesticides include: herbicides, insecticides, fungicides, rodenticides, pediculocides and biocides. Some of them contain in their structure metals (some carbamates, copper sulfate, arsenicals, granosan). Their use reduces biodiversity and nitrogen fixation, contributes to pollinator decline, destroys habitat especially for Birds and threatens species. Pesticides may cause acute and delayed health effects in those who are exposed. Carbamates cause reversible carbamylation of acetylcholinesterase. This leads to accumulation of acetylcholine at cholinergic neuro-effector junctions, which causes muscarinic and nicotinic effects and impaired CNS function. Acute exposure of the organism on copper sulfate caused anaemia, capillary damage, brain damage and depression. Copper (II) sulfate denatures proteins. Arsenicals inhibit sulfhydryl enzymes; uncouple oxidative level phosphorylation and inhibit pyruvate and  $\alpha$ -ketoglutarate dehydrogenase systems [3, 4]. Strong evidence also exists for other negative outcomes from pesticide exposure including neurological birth defects, fetal death and neurodevelopmental disorders. Some pesticides are carcinogenic, mutagenic or toxic to reproduction [2].

Adverse effects of pesticides on endocrine system and reproduction are of interest to a large number of scientific profiles (toxicologists, biologists, agronomists and epidemiologists).

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## CP4. EFFECTS OF METAL CONTAINING CYANIDES ON HUMAN HEALTH

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The present report discussed modern and actual problem concerning the effects of metal containing cyanides on Human health.

According to the definition the cyanide is any chemical compound that contains the cyano group ( $C\equiv N$ ), which consists of a carbon atom triple-bounded to a nitrogen atom. Some of them contain metals in their structure. The metal containing cyanides are: Sodium cyanide ( $NaCN$ ); Potassium cyanide ( $KCN$ ); Calcium cyanide ( $Ca(CN)_2$ ); Copper cyanide ( $CuCN$ ); Potassium silver cyanide ( $KAg(CN)_2$ ); Sodium ferrocyanide ( $Na_4Fe(CN)_6$ ); Potassium ferrocyanide ( $K_4Fe(CN)_6$ ); Potassium fericyanide ( $K_3Fe(CN)_6$ ); Sodium nitroprusside ( $Na_2[Fe(CN)_5NO]$ ) [1].

They are widely used in ore extracting procedures for recovery of gold and silver; base metal flotation; electroplating; photography and in the synthesis of organic and inorganic chemicals. Sodium nitroprusside is mainly used in clinical chemistry to measure urine ketone bodies [2, 4].

The effects of acute cyanide exposure are dominant by central nervous system and cardiovascular disturbances. The toxic effects of cyanides in humans are believed to result from inactivation of the enzyme cytochrome oxidase and inhibition of cellular respiration. The endocrine system is also target to cyanide intoxication. [3, 4]

The problems about the effects of the metal containing cyanides are important in modern medicine biology and toxicology.

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# SATELLITE SYMPOSIUM

## Session D. ADVANCED MATHATERIALS IN BIOLOGY AND MEDICINE – CHALLENGES AND PERSPECTIVES

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## DO1. NANOTECHNOLOGY AND THE CHALLENGES OF BRAIN TUMORS

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Brain tumors include a wide variety of neoplasms that can be primary or metastatic. The primary brain tumors are thought to be derived from glial cells or their progenitors and are generically classified as gliomas. 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. It has been estimated that one in every 5 000 people in their 60s develop brain tumors. This is the 3<sup>rd</sup> leading cause of death from cancer in persons aged 20 to 39 and also the most common solid tumors in children. Gliomas of astrocytic, oligodendroglial and ependymal origin account for more than 70% of all brain tumors. The common gliomas affecting the cerebral hemispheres of adults are termed “diffuse” gliomas due to their propensity to infiltrate, early and extensively, throughout the brain parenchyma. Furthermore, it has been estimated that 10% to 20% of patients with a malignant peripheal tumor develop brain metastases.

Brain cancer treatment is still one of the highest challenges in oncology. For example, survival of patients affected by glioblastoma multiforme, the most aggressive form (grade 4) of gliomas, has remained virtually unchanged during the last decades (i.e. 6-12 months post-diagnosis) despite advances in surgery, radiation and chemotherapy. The main problem in drug delivery to the brain is the presence of blood brain barrier (BBB), which limits drug penetration aiming to protect brain against toxic substances that circulate in the bloodstream. An important and long term goal of the pharmaceutical industry is to develop therapeutic agents that can be selectively delivered to specific areas in the body to maximize the therapeutic index. Different strategies have been developed for this purpose. One of the most promising among them is to attach drugs to nanoparticles and thus transport them across BBB.

**Acknowledgements:** Supported by Grant DO 02-168/2008, National Science Fund, Bulgaria.

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## DO2. ЗА НАНОТЕХНОЛОГИИТЕ И НАНОМЕДИЦИНАТА

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Нанотехнологиите са интердисциплинарна област, обединяваща в себе си химия, физика, инженерни науки, а напоследък все по-често и биология, фармация и медицина. Това е поредната крачка на научно-техническата революция, чиито постижения вече се чувстват във всички сфери на материалното производство и бита. До момента са създадени разнообразни видове наночастици (например липозоми, дендримери, нанотръбички и др.), които се различават по своите форма, размер, състав, химични и физико-химични свойства. Основните методи за получаване им включват полимеризация (*дисперсионна, емулсионна, еждуфазова*) и нанопреципитация. През последните години усилено се заговори за т.нар. наномедицина, на която се възлагат големи надежди за ранно откриване и успешно лечение на редица заболявания, включително ракови.

## DP1. NANOTECHNOLOGY THERAPIES FOR BRAIN CANCER

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## DP2. NANOTECHNOLOGY- SYNTHESIS AND FUTURE PERSPECTIVE OF METAL NANOPARTICLES

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Nanotechnology is an interdisciplinary science, studying the control of matter in an atomic and molecular scale. The use of nanotechnology can be very diverse- from medicine and electronics, to the production of biomaterials.

A perspective line in nanotechnology is the synthesis of metal nanoparticles for a variety of medical purposes – anticoagulant and anti-inflammatory substances, targeting of immuno- active and cytostatic drugs, antibiotics, anti-viral agents, gene-therapy, cancer treatment and many other fields of medical interest. The size of these particles could vary from 1 to 100nm. Due to the reduced size and dimensions of the metal nanoparticles there is a significant effect on their physical, chemical, thermal and optical properties, and makes their characteristics different from these of the bulk metal.

In the medical and pharmaceutical studies, a great place is taken from silver and gold nanoparticles. They can be synthesized through a variety of different chemical and physical methods. Nevertheless these methods could bring along problems, concerning the usage of toxic compounds. This could be solved through recently discovered 'green' techniques for synthesis of metal nanoparticles, using diverse technologies of biologically synthesizing golden, silver and other metal particles. The environmentally safe methods use biological systems, such as bacterial cultures, plants and fungi. Other than the economical significance of this discovery, biological methods provide, also a wide range of effectiveness and selectivity of the biological systems, which is also attracting interest.

Another very recent study, led to the development of the Kanzius RF therapy. It has the perspective to become an effective anti- tumor treatment. The therapy is based on the implanting of golden nanoparticles in tumor cells, which would then be exposed to radio waves with different frequency. The energy of the radio waves would heat up the nanoparticles and therefore induce apoptosis in the cancerous tissues. Unlike the conventional radio frequency therapy, the Kanzius nanoparticle treatment allows more even spread of the heat, within the cancerous tissue, without damaging normal cells. Nevertheless, the most promising potential of this therapy would be the development of a way to specifically bind the nanoparticles to the cancerous cell, which could lead to the invention of not just an effective treatment of cancer, but also to finding its cure. Therefore, certainly the role of metal nanoparticles and nanotechnology itself will become bigger and bigger in future medicine.

### References:

*NON-INVASIVE RADIOFREQUENCY ABLATION OF CANCER TARGETED BY GOLD NANOPARTICLES*

*Jon Cardinal, John Robert Klune, Eamon Chory, Geetha Jeyabalan, John S. Kanzius, Michael Nalesnik, and David A. Geller;*

*Biosynthesis of metal nanoparticles using fungi and actinomycete*

*Murali Sastry, Absar Ahmad, M. Islam Khan and Rajiv Kumar*

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### **DP3. QUATERNIZED CHITOSAN-BASED ELECTROSPUN NANOFIBROUS MATERIALS AS DOXORUBICIN CARRIERS POSSESSING ANTITUMOR EFFICACY AGAINST HELA CELLS**

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Electrospinning is an attractive and cutting edge technology for producing continuous polymer fibers with diameters ranging from a few nanometers to several micrometers, that has recently attracted a great deal of attention in the field of oncology. The use of electrospun micro- and nanofibrous materials as antitumor drug carriers is a promising approach for the targeting delivery of the antitumor drugs, because they have numerous advantages, such as improved therapeutic effect, reduced toxicity and convenience. It is well known that natural polysaccharide chitosan and its quaternized derivatives (QCh) possess good antitumor activity, as well as high antimicrobial and antimycotic properties. The combination of the advantageous properties of the antitumor drug doxorubicin hydrochloride (DOX) and the biological properties of QCh is a promising strategy for the preparation of hybrid nanofibrous materials suitable for local tumor treatment.

The successful preparation of continuous fibers containing both QCh and DOX was achieved by one-step electrospinning of mixed solutions of QCh, poly(L-lactide-co-D,L-lactide)(coPLA) and DOX.

It was found that the DOX release process was diffusion-controlled. *In vitro* cell viability studies revealed that the novel QCh/coPLA/DOX mats exhibited high cytotoxicity against HeLa cells similar to that of free DOX. A significantly reduction in the HeLa cell viability was observed when cultured on QCh/coPLA mats. The observed effect was mainly due to induction of apoptosis in the cells which is confirmed by fluorescence microscopic observations of AO and PI double-stained cells. Therefore these nanofibrous materials have excellent potential for the treatment of cervical tumor, which remains a critical public health problem.

**Acknowledgements:** Financial support from the Bulgarian National Science Fund (Grant DO-02-164/2008) is gratefully acknowledged.

### **DP4. ANTITUMOR ACTIVITY OF QUATERNIZED CHITOSAN-BASED ELECTROSPUN IMPLANTS AGAINST GRAFFI MYELOID TUMOR**

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Nanofibrous implants containing quaternized chitosan (QCh), poly (l-lactide-co-d,l-lactide) (coPLA), and the antitumor drug doxorubicin (DOX) were fabricated by electrospinning. The surface chemical composition and the morphology of the implants were characterized by XPS and SEM. *In vitro* and *in vivo* experiments were performed to evaluate the antitumor activity of the

tested implants on Graffi myeloid tumor in hamsters. In vitro cell viability studies demonstrated that QCh- and DOX-based implants exhibited high cytotoxicity against Graffi tumor cells (primary cultures from Graffi myeloid tumor in hamster) and their in vitro antitumor efficacy was similar to that of free DOX. The observed effect was mainly due to induction of apoptosis in the cells. The local application of the implants containing both QCh and DOX in the tumor tissue showed high anti tumor efficacy in vivo and was better tolerated as compared to free DOX. The implants efficiently inhibited the growth of Graffi tumor in hamsters with minimum weight loss. The experiments in which the QCh/coPLA/DOX implants were inserted locally into the tumor site postoperatively after the tumor extirpation, showed a decrease of the percentage of recurrence in the operative field and of metastases in the regional lymph nodes and an increase of the animal survival rate.

The present study demonstrates that when formulated as a nanofibrous implant, the antitumor efficacy of DOX can be significantly increased and the side effects can be reduced when it is used together with quaternized chitosan (QCh), most likely because of a synergistic action of DOX and QCh. Therefore, this type of nanofibrous implants may be considered promising as drug delivery system for local application in the treatment of solid tumors.

**Acknowledgements:** Financial support from the Bulgarian National Science Fund (Grant DO-02-164/2008) is gratefully acknowledged.

### DO3. AMORPHOUS CALCIUM PHOSPHATE FOR BI-PHASE CERAMICS PREPARATION

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Mono and bi-phase calcium-phosphate ceramic materials and the related biological activity are detailed studied. Practically insoluble mono phase bio-ceramics, built up from dense hydroxylapatite, do not actively participate in the process of bone remodeling, however at contact with body fluids they cooperate for the formation of a surface layer from bone-like apatite. In contrast, the bi-phase bio-ceramics are bioresorbable, they dissolve and stimulate in this way the growth of a new bone.

The one of main characteristic of bio-apatites that build the hard bone tissue is the content of microelements, e.g.  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{F}^-$ ,  $\text{CO}_3^{2-}$ , etc. Among them the bioactive Mg plays an important role for the formation and development of the bone tissue.

The process of biomineralization and phase transformation of amorphous calcium phosphate (ACP) to be used for preparation of bi-phase Mg modified calcium phosphate ceramics was studied at the case of three differing in composition simulated body fluids (SBFs) Two SBFs (SBFc and SBFr), differing in their  $\text{HCO}_3^-$  and  $\text{Cl}^-$  ion contents, as well as amino acid (Glycine) modified SBFg were used in the maturation studies.

It was found the maturation process leads to changes in the chemical compositions both of ACP and the maturation solution and to transformation of ACP into poorly crystalline apatite in the all studied cases. Mg content was increased in the solid phases with a maturation time due to its easy incorporation in the structure of poorly crystalline apatites. Glycine was increased the dissolution of ACP due to its ability for  $\text{Ca}^{2+}$  ions complexation.  $\text{HCO}_3^-$  ions stimulate  $\text{CO}_3^{2-}$  for  $\text{PO}_4^{3-}$  substitution in the structure as well as co-crystallization of  $\text{CaCO}_3$ . The rate of ACP phase

transformation was increased with the Glycine and  $\text{HCO}_3^-$  content, the effect of Glycine was more pronounced.

Magnesium modified ACP samples were sintered and bi-phase ceramics consisting of Mg-substituted- $\beta$ -tri-calcium phosphate and hydroxyapatite in different ratios were obtained.

**Acknowledgements:** The authors thank the National Science Fund of Bulgaria (Grants No DTK 02-70/2009 and DCVP-02/2/2009) for the financial support.

## **DO4. POROUS CALCIUM PHOSPHATES CERAMICS PROSPECTIVE AS BIOMATERIALS**

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Nano-sized calcium phosphate based ceramics possessing an interconnecting porosity network are of continuous interest in the field of the biocompatible materials as they could be used for bone repairing, reconstructive and remodeling applications. Investigations show that the increased specific surface area and pore volume of the biomaterials increase their bio-resorption ability as well they stimulate the new bone formation.

The biomimetic crystallization of nano-sized calcium phosphate precursors was studied by application of different techniques - fast mixing of the initial solutions, drop-wise precipitation, and ultrasonic. In the all cases the reaction quantities of  $\text{CaCl}_2$  and  $\text{K}_2\text{HPO}_4$  were dissolved in modified Simulated Body Fluids (SBF) and the pH of the reaction system was varying from 8 to 11. The precipitation medium was salt solution (SBF), buffer (Ammonia buffer pH 10 and Glycine buffer pH 8,9 and 10) system, mixed organic (propylene glycol)-inorganic solution, oil (olive)-in-water emulsion or gelling (Xantan Gum) system. The effect of all these variations on the composition, crystal size and morphology of the precipitated solid phases was followed.

It was found that in the all studied cases X-ray amorphous calcium phosphate or poorly crystalline carbonate apatite with Ca/P ratio 1.4 – 1.6 and mineral composition close to those of the hard bone tissues was precipitated.

A procedure for preparation of nano-sized porous calcium phosphate ceramics was proposed. Natural polysaccharide (Xantan Gum) was used as a pores forming material. The precursors sintering were done at 1000°C after two steps lyophilization.

Bi-phase ( $\beta$ -TCP and HA) calcium phosphate nano-sized porous ceramics with separated well-shaped round seeds were prepared. Their morphology strongly depended on the applied procedure.

**Acknowledgements:** The authors thank the National Science Fund of Bulgaria (Grants No DTK 02-70/2009 and DCVP-02/2/2009) for the financial support.

## **DO5. ION MODIFIED CALCIUM PHOSPHATE CERAMICS**

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Ion modified calcium phosphate-based ceramics are designed to simulate the mineral composition of the hard bone tissues or to strengthen some specific biologically important behaviour. Thus the biological active Mg plays an important role in the formation and growth of the hard bone tissue and the Zn is extremely important mediator for more than 200 enzymes.

The present study aims to examine the effect of  $Mg^{2+}$  or  $Zn^{2+}$  ion substitutions on the phase composition of calcium phosphate ceramics, their morphological and structural changes. Chemical, XRD, FTIR and SEM methods have been applied for their characteristics.

Amorphous ion modified calcium phosphate precursors were precipitated in simulated body fluids modified by different amounts of  $Mg^{2+}$  or  $Zn^{2+}$  ions ( $Me^{2+}/(Me^{2+}+Ca^{2+})$  was 0, 0.03, 0.07 and 0.13 ) at pH 8. Modified calcium phosphate ceramics with (Ca+Me)/P ratio of 1.3 - 1.4 were obtained after precursor lyophilization and sintering at 1000°C. Stabilization of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) structure occurs in the all cases of  $Zn^{2+}$  ions substitution for  $Ca^{2+}$  ions and formation of mono phased zinc- $\beta$ -TCP ceramics with sizes (500–5000 nm) and idiomorphic crystals (Fig.1a) was registered. In the case of  $Mg^{2+}$  ions substitution for  $Ca^{2+}$  ions the concentration of  $Mg^{2+}$  ions is a crucial for the stabilization of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) structure. Mono phased magnesium- $\beta$ -TCP ceramics with smaller size (100-500 nm) and spherical grains (Fig. 1b) were prepared when the  $Mg^{2+}/(Mg^{2+} + Ca^{2+})$  ratio was 0.13. When this ratio was smaller (0.03 and 0.07) bi-phased ceramics consisting of magnesium- $\beta$ -TCP and hydroxiapatite (HA) were obtained.

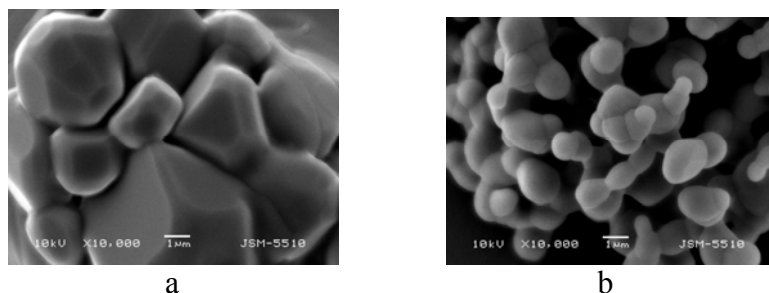


Fig.1. SEM images of ion-modified mono-phased calcium phosphate ceramics:  
a) zinc- $\beta$ -TCP; b) magnesium- $\beta$ -TCP

**Acknowledgements:** This work is financially supported by the Bulgarian Ministry of Education, Youth and Science under Projects DTK 02-70/2009 and CVP-09-0003.

## DO6. SYNTHESIS AND CHARACTERIZATION OF POLYMERIC HYDROGELS BASED ON NATURAL POLYSACCHARIDES AND GELATIN FOR BIOMINERALIZATION

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Polyelectrolyte complexes (PEC) are formed through the electrostatic attractions between two oppositely charged polyelectrolytes mixed in an aqueous solution. Chitosan is the only natural polysaccharide with a cationic nature and it is actively used for PEC formation as for example with other natural negatively charged polysaccharides as alginate, xanthane, etc. Gelatin is well known to form physically crosslinked hydrogels via non-covalent associative interactions that bring the



polymer chains together into a stable junction. Combining PEC with physically crosslinked gelatin network result into entirely natural materials based hydrogels with heterogeneous structure that is strongly influenced by a change in temperature, pH, the addition of salts, etc.

The aim of the study is to create such three dimensional heterogeneous networks and to study their properties such as mechanical properties, swelling kinetics and morphology. A variety of PEC–gelatin hydrogels were obtained by changing the complexation conditions, such as solution pH, polymer concentration, complexation time, and mixing ratio.

These hydrogels were further on used for crystallization of calcium phosphates through adsorption and diffusion of  $K_2HPO_4$  and  $CaCl_2$  solutions. The influence of the solutions components and reaction parameters on the composition, size and morphology of the crystallizing solid phases was determined.

**Acknowledgements:** This work is financially supported by the National Center for Advanced Materials UNION, № DO-02-82/2008 and Project DTK 02-70/2009 both projects funded by the Bulgarian Ministry of Education, Youth and Science.

## **DO7. DOUBLE NETWORKS OF POLY(2-ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID)-POLYACRYLAMIDE AS MATRICES FOR CALCIUM PHOSPHATE CRYSTALLIZATION**

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Double networks (DN) are a specific type of interpenetrating polymer networks with a very good mechanical performance of their hydrogels. Recently, we have observed a phase-separated morphology of DN made by polyacrylamide and polysulfobetaine with domain size in the nanometer scale which exact value strongly depended on DN composition [1]. In the present study we aim to explore this specific DN morphology in order to induce crystallization of calcium phosphates in the nanosized domains and to control this process via the DN composition.

PAMPS films have been prepared via free radical polymerization at 60 °C, and were further swelled into 1 M, 2 M, or 3 M acrylamide solutions containing also small quantities of crosslinking agent and initiator (for both networks N,N'-methylene-bis-acrylamide was used as a crosslinking agent). Then the PAAm network was obtained *in situ* after polymerization at 60°C for a few hours. Two neat networks from PAMSP and PAAm were used as referent samples. The microhardness of thus obtained films was measured and their swelling kinetics in both pure water and 0.15 M NaCl was determined. The elastic modulus of the obtained hydrogels was obtained by using the Hertz's theory. The DN samples were used for *in situ* formation of calcium phosphates by using two alternative ways, namely, by swelling into simulated body fluids and by the sequential diffusion of  $HPO_4^-$  and  $Ca^{2+}$  ions. In both cases calcium phosphates were obtained as evidenced by scanning electron microscopy and X-ray diffraction.

**Acknowledgements:** This work is financially supported by the Bulgarian Ministry of Education Youth and Science under Projects DTK 02-70/2009 and DO-02-82/2008.

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## DO8. GELATIN MICRO- AND NANOCAPSULES AS A TEMPLATE FOR CALCIUM PHOSPHATES CRYSTALLIZATION

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Biomineralization is the process that builds up the bones, teeth, shells etc. in nature. Many efforts have been done in order to prepare organic-inorganic hybrids similar to these essentials of life in laboratory conditions utilizing the biomimetic approach. Part of these efforts has been devoted to the replacement of the natural bones with gelatin-calcium phosphates hybrids aiming at e.g. the creation of bioprotheses.

The good biocompatibility of gelatin makes it an appropriate material for biomedical applications. In particular, gelatin capsules prepared by some of the common methods as w/o emulsion and coacervation, etc., are widely used in the pharmaceutical industry. In the present study gelatin capsules were prepared by applying the sonochemical method – a comparatively new method which has advantages as an easy performance and very high purity of the obtained capsules because it avoids the use of non-solvents, oil phase and surfactants. The gelatin micro- and nanocapsules were used as an environment for calcium crystal growth in an aqueous solution. The capsules promote a different crystal growth because of the confinement in the particle inner cavity.

The calcium phosphates crystallization into gelatin capsules was performed at different concentrations of the precursors and different pH values. The influence of preparation conditions on the final material properties was followed by scanning (SEM) and transmission electron microscopy (TEM). X-ray diffraction (XRD) measurements were used to characterize the calcium phosphates crystalline structure.

**Acknowledgements:** This work was financially supported by the Bulgarian Ministry of Education, Youth and Science under Projects DTK 02-70/2009 and DO-02-82/2008.

## DO9. BONE IMPLANTS – OLD PROBLEMS AND NEW PERSPECTIVEES

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Bone disease is a serious health condition that directly impacts on the quality of life of sufferers, particularly among the aged. When an area of damaged bone is too large for self-repair, the damaged bones must be repaired by using alternative materials. Autografts (the gold standard for bone replacement), which are transferred from healthy parts of the bones of the same patient, are widely used because they show high performance. However, there are problems related to a severely limited amount of tissue being available and there is additional damage to the body

because the bone tissue is extracted from the patient. Although allografts, which are transferred from other people, are also used, they have problems related to not only limited availability but also with foreign body reactions and infections. As a result there is an impetus for the development of artificial bone substitute materials that do not damage healthy tissue, do not pose any viral or bacterial risk to patients, and can be supplied at any time, in any amount.

Several biomaterials (metals, calcium phosphate ceramics, bioactive glasses, polymers, composites) have been developed with more or less clinical success. However, artificial materials implanted into bone defects are generally encapsulated by a fibrous tissue and became isolated from the surrounding bone. That is why, in orthopedic applications there is a significant need and demand for the development of a bone substitute that is bioactive and exhibits material properties (mechanical and surface) comparable with those of natural, healthy bone. Tissue engineering approaches have recently been devised to repair large bone losses. This technology takes the advantages of the combined use of cultured living cells and 3D scaffolds to deliver vital cells to the damaged site of the patient.

**Acknowledgements:** This study was supported by Grant DTK-02-70/2009, National Science Fund, Sofia, Bulgaria.

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## DO10. AN ANIMAL MODEL IN RABBITS FOR BIOCOMPATIBILITY TESTING OF BIOMATERIALS IN BONES

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The most important aspect in the development of new (biodegradable) biomaterials is the experimental and clinical testing for biocompatibility. Development of an optimal interface between bone and orthopedic or dental implants has taken place for many years. In order to determine whether a newly developed implant material conforms to the requirements of biocompatibility, mechanical stability and safety, it must undergo rigorous testing both *in vitro* and *in vivo*. The use of animal models is often an essential step in the testing of implants prior to clinical use in human. Animal models allow the evaluation of materials in loaded or un-loaded situation over potentially long time duration. Various animal species are used for these biocompatibility tests, such as dogs, sheep, goats, pigs or rabbits. At least four rabbits 5-6 month aged, should be used for each treatment. Long term implantation periods are given as 12, 26, 52, and 78 weeks. The rabbit is one of the most commonly used animals for medical research. The

International standard for the biological evaluation of medical devices recommends a maximum of 6 implants (3 test and 3 control) per rabbit. They are with cylindrical shape – 2 mm in diameter and 6 mm in length. Even though rabbits may be the least similar in bone structure and properties to the human but there are some similarities in the bone mineral density and subsequently the fracture toughness of mid-diaphyseal bone between rabbits and humans. Rabbits are commonly used for screening implant materials prior to testing in a larger animal model. This is in part due to ease of handling and size, as well as they reach skeletal maturity shortly after sexual maturity.

Main observations done in biomaterial testing are:

- General behavior of the animal (body weight, mobility, loading or non-loading, etc.)
- Biomechanical integration of implants (push out, pull out test)
- Radiographic
- Histological (general, morphometric)
- Analysis of elements (macro- and microelements)
- Biochemical (serum markers of bone metabolism: bone-specific phosphatase, calcium, osteocalcin, carboxyterminal propeptide of type I procollagen PICP), crosslinked carboxyterminal telopeptide of type I collagen (PINP)

Basing on the above observations the rabbit has been already successfully applied for several studies related to testing biomaterials.

While animal models may closely represent the mechanical and physiological human clinical situation, it must be remembered that it is only an approximation, with each animal model having unique advantages and disadvantages.

**Acknowledgements:** The work was supported by a grant from the Bulgarian Ministry of Education, Youth and Science (project DTK 02/70).

## **DO11. BIOCHEMICAL INDICES IN RABBITS WITH BONE IMPLANTS**

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It is now widely recognized that calcium phosphate ceramics such as hydroxyapatite or tricalcium phosphate are suitable bone substitutes in orthopedic, reconstructive and maxillofacial surgery because they have good biocompatibility and extensive bone conductivity. However, some problems with these ceramics have been reported. A major problem is difficulty of making them fit in with the bone surface and of shaping them in a proper form before or during operation. Recently, calcium phosphate cement attracted much attention as a new bone substitute which would overcome practical problems associated with ceramics.

Serum concentrations of biochemical markers of bone metabolism (osteocalcin, OC; total alkaline phosphatase (TAP), bone-specific alkaline phosphatase, BAP; calcium and phosphorus, were determined in rabbits with bone implants. Three types of calcium phosphate compounds, Zn modified  $\beta$ -tricalcium phosphate, Mg modified  $\beta$ -tricalcium phosphate and a mixture of hydroxyapatite and  $\beta$ -tricalcium phosphate, mixed with hyaluronic acid, were applied in rabbit bone defects. Three bone defects (2 mm/ 6mm) were created in the distal end of a femur. 19 skeletally mature male New Zealand rabbits, weighted 3-3.5 kg, were used for intraosseous implantation. Rabbits were assigned into the following groups: 1<sup>st</sup> group - control (with bone defects without any implant), 2<sup>nd</sup> group – bone defects filled with Zn-calcium phosphate, 3<sup>rd</sup> group - bone defects filled with Mg-beta-tricalcium phosphate and 4<sup>th</sup> group – bone defects with a mixture of hydroxyapatite and beta-tricalcium phosphate. Serum samples were obtained before surgery and at the 1<sup>st</sup> and 14<sup>th</sup> week after the surgery. Some deviations in the concentrations of BAP and OC

were observed while the concentrations of Ca and P were not changed in all groups. The levels of BAP were slightly increased and the level of OC was non-significantly reduced at the 14 week post operation compared to the levels at the 1st week. The concentration of TAP was slightly changed. These observations were found in all groups. There were no significant differences in the concentrations of the studied serum biochemical markers among the groups.

In our preliminary study we observed that calcium phosphate compounds applied do not have a negative influence on biological response of organism. Measurements of specific and non-specific biochemical markers of bone metabolism could be useful for evaluation of the biocompatibility of the studied compounds.

**Acknowledgements:** The study was funded by the Bulgarian Ministry of Education, Youth and Science (project DTK 02/70).

## DO12. NANOMETRICAL BI-PHASE CALCIUM PHOSPHATE AND VITALITY OF CELL TEST

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Synthetic calcium phosphates in form of blocks, discs or granules have been widely used for medical and dental applications such as bone augmentation, repairing and reconstruction. Calcium phosphates have been recently tested as composite scaffolds for bone tissue engineering and cellular therapy.

The purpose of this study is to follow the effect of dry mechanochemical treatment of sintered bi-phase calcium phosphate ceramic (BCPC) consisting of HAP (hydroxyapatite) and  $\beta$ -TCP (tricalcium phosphate). The phase modification and crystal size are studied by X-ray, IR and SEM.

It was found that the sintered at 1100°C, grinding materials was with about 70-80 nm crystal sizes of hydroxyapatite phase. The particles of the  $\beta$ -TCP phase were transformed into amorphous phase. Mechanical grinding is useful method to obtain nano biphasic mixtures of HAP/  $\beta$ -TCP.

The cell vitality was performed using MTT test, neutral red assay cytotoxicity assay and crystal violet staining. The cells were cultured for 72 h in media where the materials were incubated for 4 h.

*In vitro* cell culture test demonstrate that the milling modified materials is more active than the control one. No cytopathological changes were observed.

Nanosized BCPC was used as component of anisotropic composite material (nanosize BCPC, chitozan and poly L-lactid).

It was found the particles were randomly dispersed in the composite scaffold.

**Acknowledgements:** This work is financially supported by the Bulgarian Ministry of Education, Youth and Science under Projects CVP-09-0003 and DTK 02-70/2009.

## DO13. FUNDEMENTAL APLICATION OF TITANIUM AND TITANIUM ALLOY

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Titanium metal was first discovered by the English chemist William Gregor in 1791 in the black magnetic sand ilmenite, and the metal was named "titanium" after the titans of Greek mythology, a symbol of power and strength. Titanium is the fourth-most-abundant metal in the Earth's crust, the other three being aluminum, iron, and magnesium. Titanium has low density and high strength, good corrosion and erosion resistance to different media, good oxidation resistance, and moderate strength at high temperatures, making it attractive for industrial applications. It has a number of features that distinguish it from other light metals.

Basically, titanium and titanium-based alloys can be classified into  $\alpha$  type (HCP: hexagonal closed-packed crystalline structure), near  $\alpha$  type, ( $\alpha+\beta$ ) type, and  $\beta$  type (BCC: body centered-cubic crystalline structure) alloy groups. Alloying elements added to titanium are divided into two groups: alpha ( $\alpha$ ) stabilizers and beta ( $\beta$ ) stabilizers. The properties of metals are essentially based on the metallic bonding of the atoms in the crystal lattice.

Titanium alloys primarily stand out due to two properties: high specific strength and excellent corrosion resistance. This also explains their preferential use in the aerospace sector, the chemical industry, medical engineering.

There are three main reasons to use titanium as a structural material: excellent resistance, high specific strength, and exceptional biocompatibility. Therefore, titanium and its alloys have primarily found application in the chemical and aerospace industry, after that electrochemical, automotive industry, architecture, and as well as in biomedicine.

Under normal conditions in human body fluid, i.e. a solution of about 0.9% NaCl, the pH value amounts to about 7.4. Changes in the pH value can be caused by surgery, resulting in a rise to 7.8 and followed by a drop to 5.5. After a few days the normal value of 7.4 is re-established. In this medium the most corrosion resistant materials are titanium and its alloys, niobium, and tantalum, followed by wrought and cast vitallium, and by stainless steel. For this reason biomaterials must fulfill the following requirements: a) corrosion resistance; b) biocompatibility; c) bioadhesion (osseointegration); d) processability (casting, deformation, powder metallurgy, machinability, welding, brazing, etc.); e) availability (low prices)

The first successful heart transplantation on December 3rd, 1967, at the Groote-Schur hospital in Cape Town, South Africa, the implantation of the first artificial heart into the body of Robert Tools. Apart from many soft tissues and plastic tubing, the metallic parts of the artificial heart, like connectors, valves, etc., are manufactured from titanium.

This pioneering work underlines the important progress titanium and its alloys have made as surgical implant materials in medical engineering within the last 30 years. The excellent compatibility with the human body is regarded as a key property for the choice of titanium: Titanium is generally perceived as the biocompatible metallic material. Furthermore, titanium is extremely resistant to corrosion from body fluids, and is compatible with bone and living tissue, and is elastically deformable as thin foil material. Thus, pure titanium combines many of the attributes desirable for heart pacemaker cases and as the carrier structure for replacement heart valves.

Hard tissues are often damaged due to accidents, aging, and other causes. It is a common practice to surgically substitute the damaged hard tissues with artificial replacements. Depending on the regions in which the implants are inserted and the functions to be provided, the requirements of different endoprosthetic materials are different.

Because of the aforementioned desirable properties, titanium and titanium alloys are widely used as hard tissue replacements in artificial bones, joints, and dental implants. As a hard tissue replacement, the low elastic modulus of titanium and its alloys is generally viewed as a biomechanical advantage because the smaller elastic modulus can result in smaller stress shielding.

One of the most common applications of titanium and its alloys is artificial hip joints that consist of an articulating bearing (femoral head and cup) and stem. The articulating bearings must be positioned in such a way that they can reproduce the natural movement inside the hip joints whereas secure positioning of the femoral head in relation to the other components of the joint is achieved using the stem. The hip stem is anchored permanently to the intramedullary canal of the femur. The cup, which is the articulating partner of the femoral head, is used for fixation by reaming out the natural acetabulum to fit the design. Titanium and titanium alloys are also often used in knee joint replacements, which consist of a femoral component, tibial component, and patella.

Titanium and titanium alloys are common in dental implants, which can be classified as subperiosteal, transosteal, and endosseous according to their position and shape. The prosthesis is secured on posts or abutments that penetrate the mucosa into the oral cavity. They are the most commonly used implant types and can be used in almost any situations as single implants to replace one missing tooth as well as in cases of partial and total edentulism. The most commonly used endosseous implants are root-forming analogs. Most of the dental implants are placed according to the “osseointegration” concept that allows dental implants to fuse with bones. Surface modification technologies, such as grit blast, chemical etching, and plasma spraying are often utilized to improve the osseointegration ability of titanium dental implants.

The movement of an artificial hip joint produces billions of microscopic particles that are rubbed off during motions. These particles are trapped inside the tissues of the joint capsule and may lead to unwanted foreign body reactions. At the boundary layer between the implant and bone, these interfere with the transformation process of the bone leading to osteolysis. Hence, the materials used to make the femoral head and cup play a significant role in the device performance. Since the advent of endoprosthetics, attempts have been made to reduce wear by using a variety of different combinations of materials and surface treatments. The search for materials with high wear resistance and surface modification technologies to improve the wear resistance of existing clinical materials continues to attract a lot of scientific interest.

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## DO14. DEFENSINS – THE NOVEL PUTATIVE ANTIRETROVIRAL TOOL

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Defensins are a family of small cationic, antibiotic peptides that contain six cysteines in disulfide linkage. The peptides are abundant in phagocytes and small intestinal mucosa of humans and other mammals. They contribute to host defense against microbes and viruses – so far known effect against flu, herpes and HIV and are considered an important element of innate immunity. The interest to defensins in HIV field is conferred by their antiviral effect possibly due to a barrier function in viral entry process. Bioengineered and synthetic defensins seem potentially useful as prophylactic and therapeutic agents in HIV infections.

Here we present the first in Bulgaria in vitro study of effect of two defensins – coded 1 and 3 and synthesized in the Dept. of Life Sciences in University of Trieste, Italy. Cytotoxicity was first checked by MTT test (Mosmann T., 1983, Montefiori, D. et al., 1988), expressing survival of MT-4 cells after treatment with different concentrations of defensins 1 and 3 – defensin 3 showed almost no cytotoxicity. Both defensins were nontoxic in concentrations 0,18 – 0,37  $\mu$ M. Additionally, RT activity was also measured – both demonstrated inhibiting activity on HIV-1 replication. To reveal the mechanism of action, gp120 concentrations were measured immediately after the contact with HIV-1 stock for 1h in ice bath, and then after 72h virus replication. Decrease of gp120 after the contact with the virus correlated well to the gp120 concentration in supernatants, i.e. after 72h virus replication thus pointing to interaction between gp120 and the defensins as a mechanism. Studies are extended with another five synthetic defensins.

## **DP5. EXPERIMENTAL NEUROBIOLOGY: ARTIFICIAL NERVE DEVELOPING**

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Neural tissue has been regarded as having poor regenerative capacity but recent advances in the growing fields of tissue engineering and regenerative medicine have opened new hopes for the treatment of nerve injuries and neurodegenerative disorders. Adipose tissue has been shown to contain a large quantity of adult stem cells. These cells can be easily harvested with low associated morbidity and because of their potential to differentiate into multiple cell types, their use has been suggested for a wide variety of therapeutic applications (Erba *et al.*, 2010). Many experimental *in vivo* studies have indicated that Schwann cells are key facilitators of peripheral nerve regeneration, but their clinical therapeutic potential may be limited. Recent advances suggest that stem cell therapy could one day be used to treat nerve traumas. In a recent report Kingham and collaborators have shown how adult stem cells can be differentiated into a Schwann cell phenotype, characterised by expression of glial cell proteins and promotion of neurite outgrowth (Kingham *et al.*, 2011).

The development of new cell culture models which mimic the *in vivo* regeneration environment will be of help for better understanding the functional benefits of these cells. Recently, the research team of Prof. Douglas Smith from Penn Center for Brain Injury and Repair has created a three-dimensional neural network, a living conduit in culture, which can be transplanted en masse to an injury site. In truth, Smith and his colleagues have created formations made from axon bundles, engineered directly in the lab, which after grow are transplanted into the area that needs repair, and then help the host tissue to regenerate itself. A small portion of rat sciatic nerves was removed, and then the same animal underwent surgery to have the lab-grown axon bundles placed inside them. Some 16 weeks after the operation, the area that had been operated showed unexpected signs of improvement, and the researchers noted that the transplanted axon bundles and the damaged ones seemed to have intertwined, creating a new form of axonal regeneration.



In summary, the technology of artificial nerve development is still in its infancy, but contains the potential for a radical shift in brain/electronics interfaces. In the short term, this technology could help people who suffer from diseases like schizophrenia, by releasing the neurotransmitters needed to regulate the out-of-control nerve firings associated with those diseases. In the long term, as the technology becomes smaller, cheaper, and able to receive neurotransmitter signals as well as send them, these artificial nerves could be used to create bionic brain prostheses for stroke victims, or even serve as the intermediate between our biological brains and electronic computers.

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