Session 9. METAL IONS AND PLANTS

POTENTIAL HEALTH BENEFITS OF POLYPHENOL POLYMERS FROM CINNAMON

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The causes of glucose intolerance and diabetes are not clear but diet is known to play a central role. Excess consumption of simple carbohydrates and fats have definite negative effects but certain herbs and spices have been reported to improve glucose tolerance and may prevent the progression to diabetes. Aqueous extracts of cinnamon were shown to increase the activity of insulin more than 20-fold in an epididymal fat cell assay. Known components of cinnamon had negligible effects on insulin activity. The active components of cinnamon were isolated and shown to be polyphenol polymers. The trimer had a molecular weight of 864 and the tetramer had a molecular weight of 1152 with larger polymers of undetermined molecular weight. All of these had insulin potentiating activity and the bioactivity was not related to the length of the polymers. Fractions containing polyphenol polymers displayed activity similar to that of insulin in 3T3-L1 fat cells during glucose uptake, glycogen synthesis and phosphorylation of the insulin receptor. The mechanism of action of these polymers involves the activation of insulin receptor kinase and the inhibition of insulin receptor phosphatase leading to maximal phosphorylation of the insulin receptor and thus greater insulin sensitivity. These polymers also displayed significant antioxidant activity and appeared to display activity similar to or greater than those of commercial antioxidants. In summary, fractions from cinnamon high in polyphenol polymers display beneficial effects on insulin function and also antioxidant activity. These data suggest that polymers found in cinnamon may be beneficial in the control of glucose intolerance and diabetes.

THE STUDY OF EFFECT OF CALCIUM CHANNEL BLOCKER ON THE CHANGE OF FUNCTIONAL ACTIVITY OF PLANTS

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The use of novel high sensitive apparatus — laser interference auxanometer and CO_2, O_2 and H_2O steam simultaneous analyzers made it possible to study response reactions of both the whole plant and its separate organ (leaf) on effect of biologically active compounds. Wheat, buckwheat, rice, oat, sugarcane and geranium plants were used for study of the action of calcium channel blocker, verapamil, on their functional activity. It was shown that high verapamil concentrations suppressed uptake of CO_2, O_2 evolution and transpiration in leaves in the light. Low verapamil concentrations slowed down the above mentioned processes at prolonged actions (few days) and stimulated them at short actions (few hours). Verapamil slowed down after short stimulating effect or stopped (depending on concentration) growth of plants. The growth rate decreased to zero or to a certain constant level. In experiments with low concentrations of verapamil increase in growth rate was observed after its significant decrease for 2.0–3.0 h (i.e. restoration process took place). At addition of antioxidant ambiol to plants, treated with verapamil, the plant growth rate was restored more rapidly than without addition of ambiol. Susceptibility of plants to verapamil depended on their genotypic properties and growing conditions. Rice plants, grown at higher illumination and temperature, were less susceptible to effect of verapamil than those grown at low illumination and low positive temperatures. The increase in temperature in the root zone from 22°C to 32°C increased susceptibility of buckwheat plants to verapamil to a greater degree than of rice plants. Participation of calcium dependent regulatory systems in the change of functional activity of plants and their adaptive capacity under disturbance of calcium transport is considered.

ENHANCEMENT OF FUNGAL RNA AND METABOLISM BY METAL IONS AND THEIR BINDING: RT-PCR PRODUCTS EXPRESSION

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Background: The effects of metal ions on growth and toxin production by fungi has been demonstrated by Jackson, M.S. (App. And Environ. Microbiol., 55: 649–655. 1989); Cuero, R. Mycotoxins and Phycotoxins in Perspective at the Turn of the Millenium, Chapter 10: 355–361, 2001). Similarly the role of metal ions on the expression of nucleic acids has been reported by Failla, M. (Marcel Dekker, Chapter 4: 152–214, 1977), and Cuero, R. (Metal Ions in Biology and Medicine, pp. 765–767. John Libbbey Eurotex, 2000).

Aims: The aims of the present work was to determine the effects of metal ions on the genomic information in relation to fungal growth, toxin production and their binding to the metal ions (Zinc, Copper, Iron).

Methods: Different metal ions, in single or combined, (Zinc, Copper, Iron) concentrations (0–5 ppm), were used to treat fungal cultures (*Aspergillus flavus* and *Fusarium graminearum*) in liquid cultures, and incubated for different times (3–6 days) and temperatures (25–30°C). After incubation, samples were taken for analysis of RNA, cDNA, RT-PCR, fungal growth, and mycotoxin metabolite synthesis and metal ion binding. Mycotoxinz (aflatoxin and zearalenone), were analyzed by ELISA, and binding of metal ions by Electro Spray Ionization Mass Spectrometry.

Results: There were differential effects of the single metal ions on the fungal RNA, toxin synthesis, growth, and binding, depending on the type of metal ion and fungal species. However, combined ions always showed higher enhancement of RNA, RT-PCR products and mertal binding to toxins in all fungal species (>50%). Single Zinc also enhanced (>25%) *A. flavus* RNA, RT-PCR products, growth, and metal ion binding to the fungal toxin (aflatoxin). Iron and Copper had similar effects, but in *F. graminearum*. Copper, always showed the highest binding to both fungal toxins and to the aflatoxin precursor.

Conclusions: Metal ions induced genomic modification on both *A. flavus* and *F. graminearum,* thus enhancing their RNA and gene expression in relation to cellular growth, and toxin synthesis;depending on the type and concentration of metal ions, and on the fungal species. Binding of metal ions to the toxin metabolites was marked, especially by copper, thus suggesting an interactive mechanism for toxin synthesis.

SCREENING OF *BRASSICA JUNCEA* VARIETIES FOR TOLERANCE TO CADMIUM TOXICITY

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It is known that *Brassica juncea* (L.) Czern. (Indian mustard) shows a strong ability to accumulate different heavy metals (HMs) including cadmium (Cd) and can be considered as one of the most promising plant species useful for phytoremediation of soils contaminated with HMs. The aim of the present research was to study the variability of Indian mustard in Cd tolerance and to select Cd tolerant genotypes. Fifty-one Indian mustard varieties from the World Collection of the VIR (St. Petersburg) were screened for tolerance to Cd toxicity using growth pouch culture. Cadmium tolerance was determined by a tolerance index (TI) calculated as a ratio between the root length of Cd-treated and untreated 10 days old seedlings in the presence of 60 μM $CdCl_2$ in the nutrient solution. The TI varied between genotypes from 60% to 100%. The distribution of varieties according their Cd tolerance was characterised by variation coefficient of V = 11 ± 1 %. The distribution pattern did not differ from the expected normal distribution in skewness (As = –0,42) and kurtosis (Ex = –0,36). Processing the data for TI and the data for several phenotypic and economic traits of the varieties (available from the VIR database) with correlation analysis showed a significant negative correlation between Cd tolerance and plant productivity or accumulation of eicosenoic acid in seeds. Certain of varieties having an increased Cd tolerance were identified.

THE EFFECT OF MEDIUM ORGANIZATION ON THE INTERACTION OF METAL IONS WITH NATURAL PIGMENTS

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Elucidation of the mechanism of antioxidant activity of biologically active substances in living organisms represents a fundamental problem in biology and medecine. It is well known that antioxidant activity of natural flavonoids is strongly dependent on the presence of metal ions; this conclusion is based mainly on the results of studies on the flavonoid-metal ion interactions in molecular, mostly water or water-alcohol solutions. However, in biological systems these processes take place in more or less highly organized medium (in the intercellular or intracellular media, in cell organelles, etc.); hence, molecular solutions seem to be not adequate models for such studies. That is why we choosed for our research of flavonoid-metal ions interaction the

reverse micellar system; here the interaction under question takes place in the internal space (polar core) of reverse micelles, similar in its properties to the internal medium of membrane structures. Our aim was, taking quercetin (Qr) as an example, to compare its interaction with Ag^{+} and Cu^{2+} ions in water solutions and reverse micelles (in Me(H_2O/AOT/n-alkane ternary system). We used UV-VIS spectrophotometry for the control of optical properties and photon correlation spectroscopy for the determination of particle size distribution in liquid phase.

In phosphate buffer we confirmed the formation of Me-Qr complexes presumably because of the partial charge transfer from Qr to metal ion. In micellar solution, however, subsequently to the complex formation the appearance of metal nanoparticles was detected. The characteristic features of these nanoparticles were (1) high stability in liquid phase in the presence of molecular oxygen and (2) intensive absorption bands in visible region with λ_{max} = 410–440 nm (Ag) and λ_{max} =545–560 nm (Cu). Photon correlation spectroscopy measurements allowed to confirm the formation of nanosized particles and to determine their size distribution in micellar solution. It was shown that Ag particles are 2–4 nm in size. The subsequent events in the metal ions-nanoparticles-Qr system depend on the Qr to metal ions ratio. In high enough Qr concentration even the dissolution of silver nanoparticles may take place.

Our results testify to the strong influence of medium organization on the process of Qr-metal ions interaction. The formation/destruction of metal nanoaggregates in micellar system shows to the real possibility of similar processes in the living systems.

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STUDY OF INTERACTION EFFECTS OF SALINITY AND CADMIUM ON SOME PHYSIOLOGICAL ASPECTS AND ION CONTENTS OF *ZEA MAYS* L.

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Short–term studies for comparing some growth and photosynthesis responses and ion contents to NaCl (0, 10, 25, 50, and 100 mm) and $Cd(No_3)_2$ +4H_2O (0, 2.5, 5 and 10 μg L^{-1} Cd^{+2}) in seedlings of maize plants were carried out under controlled conditions. The effect of salinity on growth of plants depended on the level of salinity stress imposed. With increasing of salinity in Hoagland nutrient solution the contents of total soluble sugars, concentration of Na (in shoot and root) increased and starch contents, concentration of K (in shoot and root), leaf area, shoot and root fresh matter, total dry matter production , chlorophyll's contents relative growth rate , leaf water content per unit leaf area and unit leaf rate decreased. By addition of different concentration of cadmium to solutions with salinity, final responses were different. Cadmium and NaCl reduced their negative effects.At salinity levels 25 mm for example, total soluble sugars contents decreased with increasing of cadmium content at nutrient solution and starch content increased. These results were shown decreasing of respiration rate in these plants. In addition, the growth parameters were better than other treatments. Determination of cadmium contents shown that with increasing of salinity, the absorption of cadmium by roots and transport of cadmium to shoots decreased. In Hoagland solution with 25 mM (NaCl) and 5 μgL^{-1} Cd^{+2} the lowest Cd^{+2} content was shown. The addition of cadmium caused a partial elimination of salinity effects on roots and shoots in *Zea mays* plants.

ALGAL RESISTANCE TO HEAVY METALS

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There are many experimental data about algal adaptation to heavy metals. The result of adaptation is increasing of resistance to toxicants during the time. In the case of chronic intoxication this process can develop by selection of already existent forms in genetically heterogeneous population (genetic adaptation) or by forming of resistant cells of algae within population (biochemical or phenotypic adaptation). It is important to know the limits of algal population resistance to long-term high intensive toxic effects for hydrosphere monitoring. In present work we estimated resistance of laboratory population of green chlorococcal alga *Scenedesmus quadricauda* (Turp.) Breb. to potassium dichromate ($K_2Cr_2O_7$) as a model toxicant. After the pre-adaptation to relatively low concentrations of toxicant the algae were re-inoculated twice in medium with 10.0 mg/l $K_2Cr_2O_7$ (excluding control culture). It was revealed that the number of cells depended on initial $K_2Cr_2O_7$ concentration: the higher the toxicant concentration was, the less cell number was. So, in a 30 days maximum cell number was in pre-adapted with 0.1 mg/l $K_2Cr_2O_7$ culture. In another 30 days the cell number was the same in all samples (5–6 % of initial cell number). During the experiment photosynthetic efficiency decreased accordingly to toxicant concentration: the higher concentration was, the lower photosynthetic activity was. Living cells

were found out in cultures after three-time intoxication. They had size spectra and functional characteristics the same than a control ones. The resistant cells are suggested to relate to their constant presence in population or are the result of selection. It is need of special research for clarification of this phenomenon.

EFFECT OF HYDROFLUORIC ACID ON RECOVERY OF SELECTED TRACE ELEMENTS IN ACID DIGESTS OF PLANT AND PEAT MATERIALS

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Background: There is a growing interest in the use of peat bogs as archives of atmospheric metal deposition. However, accurate and precise analytical data are needed to provide reconstructions of atmospheric metal deposition rates. Thus, reliable analytical procedures for trace element analyses are a critical first step in any such study. Almost all analytical techniques for the determination of trace elements require solid samples to be dissolved. This digestion is commonly achieved by heating the samples with acids. Hydrofluoric acid (HF) is normally used in the acid mixture to attack silicates which are present in the samples mainly in the form of fine-grained soil dust. However, many elements such as Ca and rare earth elements form insoluble fluorides that easily precipitate and result in poor recoveries of these elements.

Aims: To develop a simple, robust and reliable analytical procedure for the dissolution of silicates in plant and peat materials followed by quantification of selected trace elements in the digestion solutions.

Methods: Three different closed-pressurised digestion devices operated at various maximum temperatures (180°C to 320°C) for the dissolution of peat and plant samples have been evaluated. We studied the use of tetrafluoric acid (HBF_4) as a possible replacement for HF in the digestion solutions. Acid mixtures contained HNO_3 with or without addition of either HF or HBF_4. Trace elements were subsequently determined by inductively coupled plasma mass spectrometry (ICP-MS).

Results: The temperature applied during digestion is not the decisive factor of the entire analytical procedure, but rather the composition of the digestion mixture. In particular, the presence or absence of HF was found to play a crucial role.

Conclusions: HNO_3 alone cannot liberate 100% of all elements during digestion of silicate containing peat and plant matrices. The conventional approach which is to add HF, dissolves siliceous matter reasonably well, but forms insoluble metal fluorides that negatively affect the quantification of many elements. The addition of HBF_4 to the digestion mixture efficiently solves this problem by simultaneously attacking the silicates while preventing the precipitation of metal fluorides.

TYPES OF ALGAL POPULATION RESPONSES TO HEAVY METAL ACTION

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An usual behavior of biological systems is three-phase response to treatment dose ("paradoxical reaction"). It means that low and high doses of factor cause a stronger response than moderate ones. We were shown earlier that nonlinear concentration-effect curve reflects a hierarchy of cell responses to increasing concentrations of damaging factor: inhibition of cell division at low concentrations; stress and adaptive increasing of resistance at moderate concentrations and premature cell division and death at high concentrations. In this work, we analyzed the dynamics of the population structure and cell functional characteristics (photosynthesis) of laboratory green alga *Scenedesmus quadricauda* (Turp.) Breb. population. Thereby, we found the main types of algal reaction to the toxicant (potassium dichromate, $K_2Cr_2O_7$) action. At low $K_2Cr_2O_7$ concentration (0.001 mg/l) the total cell number decreasing was not related to cell death. The decrease of the growth rate in this case was due to the long-term cell division inhibition in a fraction of cell population but toxicant did not have strong effect on the photosynthetic activity as compared to control culture. At moderate, seemingly inactive concentrations (0.01–0.1 mg/l) the absence of effect is caused by renewal of cell division after temporary arrest. During the arrest of cell division, the efficiency of photosynthesis decreased only slightly but restored within two days. At these concentrations toxicant induced cell stress and adaptive elevation of cell tolerance. At medium toxic concentrations (1.0–3.0 mg/l) we can observe long-term cell division inhibition and giant cells forming but such a state of the algae was reversible: giant cells rapidly resumed their division after being transferred to a toxicant-free medium. Sublethal $K_2Cr_2O_7$ concentrations did not significantly inhibit photosynthesis. At lethal concentration (10.0 mg/l) the cell division is stimulated and the small immature cells predominated in the beginning of intoxication. The toxicant caused reducing photosynthetic efficiency to a double as compared to control level. It indicated irreversible cell damage. Thus, changes of the population structure characterize its state. We can recommend using described types of reaction to the toxic action for risk assessment and biotesting.

EFFECT OF DIFFERENT GROWTH MEDIA ON UPTAKE OF TRACE ELEMENTS

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During recent years significant progress has been made in understanding the processes of uptake and pathways of ions in plants, Kinetics of nutrients in roots and their translocation to leaves. Unfortunately, because of influence of numerous factors affecting the movement of elements through soil to roots, the effects of ion concentrations in the environment on uptake and kinetics of the ions in plants have been often studied in solution culture. Meanwhile, there is no doubt that soil and water (including nutrient solutions) differ widely in bioavailability of nutrients and the ability to supply plants by micro- and macro-elements in quantities sufficient for optimal plant growth. The aim of the research was to study effect of surrounding media on the uptake of different ions by roots and transfer of the ions from roots to leaves.

Short-term tests have been performed to assess variability of element uptake by wheat seedlings grown in different water solutions (nutrient solution of Hoagland, water taken from a spring and double distilled water) and in soil. ICP-MS, instrumental neutron activation analysis and liquid ion chromatography were used to determine concentrations of more than 30 elements in plants, soil and water solutions. Multivariate statistical treatment of experimental data was used to estimate similarities and differences of element behaviour in plants grown in the media and classify the plants according to their ability to uptake the ions.

In spite of very different concentrations of elements in the experimental media, plants maintained their elemental composition at more or less determined level. However, there were specific differences in uptake of elements by roots of plants grown in different conditions and rather similar behaviour of elements in leaves. Besides, relationships between elements in experimental media and in different parts of plants were quite different. In fact, there was no similar correlation between any two elements in both any medium and all parts (roots and leaves) of the plants grown in the medium.

BIOAVAILABILITY AND INTERACTION OF ULTRA TRACE ELEMENTS

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Bioavailability and toxicity of many trace elements remains unknown. For our research we chose two such elements – scandium and thorium. Biological role of all radio-nuclides is still not clearly understood. Compared to other radio-nuclides, much less attention was given to thorium. The bioavailability and toxicity of scandium is quite unknown, though this trace element always can be detected in all living organisms.

The effects of Th and Sc on uptake and bioaccumulation of different macro- and micro-nutrients (including Sc and Th) were studied under greenhouse conditions. To assess the influence of germination conditions on element uptake and plant growth, a part of seeds of wheat *Triticum vulgare* (Vill.) Horst was treated before sowing by solution containing small amounts of Th, Sc or Th+Sc. Germinated wheat seedlings were grown for seven days in the pots filled with soil. Concentrations of 26 elements in soil, roots, leaves and seeds were determined by instrumental neutron activation analysis and ICP-MS.

The rate of germination of experimental seeds was suppressed significantly in the case where concentrations of both elements — Th and Sc — in the solution used for germination were increased simultaneously (though transfer of the germinated seedlings to soil was favourable for survival of the plants). Addition of Th to the soil resulted in significant increase of Th content in both leaves and, especially, roots of the experimental plants. Meanwhile, there was no effect of Th accumulation on the biomass of the plants. This indicates that Th is not very toxic for the plants. The similar situation was observed after increase of Sc concentration in the soil. Addition of both elements (Th and Sc) to the experimental soil resulted in the increase of concentration of the elements in the experimental plants, but level of the concentrations was less than in the experiments described above. Treatment of plant seeds before experiment by solutions of Th and Sc and addition of the elements to the soil caused significant variations in concentrations of some other elements in the experimental plants.

METAL PHYTOEXTRACTION BY CEREALS

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Recently we have shown that application of growth regulators led to sharp increase of root exudative activity of some cultural (*Zea mais* L.) and wild cereals during first stages of germination. In this work we present results obtained in experiments with *Lollium perenne* L., *Festuca rubra* L., grown on sterile sand and on soils contaminated with great quantities of Zn and Pb salts with application of previously described growth regulator FeSuc (unstechiometric mixture of Fe-succinate). Detailed analysis of amino acid content of root exudates of cereals showed that wild specie had more certain amino acids (cysteine, aspartic and glutamic acids and their amides, serine) in root exudates than cultural ones. These amino acids had more possibility for chelation due to existance of one more polar or ionogenic functional groop. Seeds of cereals were treated with FeSuc and planted on soils contaminated with salts of Zn and Pb. It was shown that during 15 days of germination quantity of metals in primary leaves increased approximately in twice and decreased in soil, especially in upper layers (on 7–10%). Amino acids in root exudates of plants may be chelate agents as an aa-amino acid can act like a bidentate ligand, forming a five-membered heterocyclic ring with suitable metal cations and in a such way increasing mobility of metal ions. Thus, it was shown that stimulation of root exudative activity by pretreatment with a growth regulator may be succesful in cleaning of soils and basicly may be a good instrument for phytoremediation.

STUDY OF HEAVY METAL STRESS ON PLANTS BY HYPHENATED TECHNIQUES

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Cucumber plants grown in nutrient solutions and artificially contaminated with nickel, were chosen as model edible plants for the investigations focusing on the determination of the chemical transport of essential and toxic heavy metals in their xylem vessels, which aimed the elucidation of the mechanism of phytotoxicity. For this purpose, "off-line" size exclusion high performance liquid chromatographic — graphite furnace atomic absorption (SE-HPLC — GF-AAS), "off-line" SE-HPLC — total reflection X-ray fluorescence (TXRF) and "on-line" SE-HPLC inductively coupled plasma atomic emission spectrometric (ICP-AES) methods were developed. By employing these methods for standard solutions containing citric acid in various concentrations (100–500 $\mu g/cm^3$) and nickel in concentration of 2 $\mu g/cm^3$ as well as for xylem sap samples, citric acid seemed to be involved in the nickel transport within the plants. Also, the role of citric acid in the Fe and Mn transport in plants could not be discarded.

AVAILABILITY OF RADIONUCLIDES FOR BARLEY PLANTS INOCULATED WITH PLANT GROWTH-PROMOTING RHIZOBACTERIA

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The aim of this study was to assess the ability of beneficial rhizosphere bacteria to accumulate radionuclides and to estimate their possible implication for uptake of ^{134}Cs by the plants from nutrient solution and contaminated soil. The studied strains of plant growth-promoting rhizobacteria *Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7, *Agrobacterium radiobacter* 10 and *Flavobacterium* sp. L30 were able to accumulate ^{134}Cs and ^{90}Sr by batch cultivation in a liquid nutrient medium. Radiocaesium was concentrated in bacterial cells to a considerably lesser extent than radiostrontium. The strain *A.lipoferum* 137 was the best for immobilization of radionuclides, having a biomass-medium concentration ratio (CR) of 560 and 6400 for ^{134}Cs and ^{90}Sr, respectively. In hydroponic culture, inoculation with the strains *A. lipoferum* 137 and *Flavobacterium* sp. L30 increased CR of ^{134}Cs in shoots of barley seedlings by 50%. When barley was grown in soil supplemented with ^{134}Cs, the effect of bacterial inoculation on uptake of ^{134}Cs by the plants varied significantly depending on the strain. *A. lipoferum* 137 decreased CR and ^{134}Cs content in roots and straw. *Flavobacterium* sp. L30 increased total ^{134}Cs in plants, probably due to an increase in the plant biomass. Parameters of the model based on three compartments (soil solution - plant - bacterium or nutrient solution - plant - bacterium) were calculated. The direct and return constants of the ^{134}Cs distribution rate between compartments were evaluated. The results showed that plant growth-promoting rhizobacteria are capable of immobilizing radionuclides and could affect the availability of radionuclides for plants after their introduction into the rhizosphere.

REDUCED HEAVY METAL LEVELS IN LEAVES OF SLUDGE TREATED TOBACCO PLANTS

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Anthropogenic interventions, such as inorganic fertilization, produced soil aggravation by heavy metals whereas organic fertilization, by means of sewage sludge application, produced conflicting results in relation to heavy metal soil pollution.

Heavy metal bioaccumulation in plants is related to species physiology and plant part, environmental conditions and essential metal antagonism, substrate's metal burden and bioavailability.

Tobacco leaves insulted by metals via substrate pollution construct a major health issue for smokers due to heavy metal toxic effects on humans.

The present work aims to compare the effects of inorganic and organic (treated domestic sewage sludge) fertilization on two Greek oriental type tobacco varieties in relation to heavy metal bioassimilation.

Two (2) tobacco varieties (I and II) were grown on soils of different pH (5.5 and 7.4), mixed with either anaerobically treated sewage sludge (pH: 7.1) at various proportions (0%, 10%, 20% and 40%) or inorganic fertilizer.

The determination of Cd, Zn, Cu, Pb and Cr was conducted in tobacco leaves by means of atomic absorption spectrophotometry (AAS).

Plants grown on acid soils presented significantly lower Cd levels in both varieties after sludge treatment in comparison to inorganic fertilizer application ($p<0.05$).

Plants grown on alkaline soils presented significantly lower Cd, Cu and Pb levels, only in variety I, after high sludge proportion treatment (20% and 40%) in comparison to inorganic fertilizer application.

Sludge proportion 40% resulted in statistically higher Zn levels in both varieties and soil pH regime.

Variety I plants grown on sludge, presented significant correlation between essential and non essential metals.

In conclusion, metal bioavailability in tobacco leaves is depended on substrate's features and variety's genetic traits, hence fertilizing practices should consider various parameters in order to protect public health.

THE COMPARISON OF PHYSIOLOGICAL RESPONSES OF *TRITICUM AESTIVUM* AND *GLYCIN MAX* TO INTERACTION OF CADMIUM AND SALINITY

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The effect of o. 2.5, 5 and 10 μg $(Cd^{+2})L^{-1}$ [$Cd(NO_3)_2 + 4H_2O$] and 0, 10, 25, 50 and 100 mM NaCl on certain parameters of photosynthesis and growth in *Glycin max* (dicot) and *Triticum aestivum* (monocot) plant shown different responses to these stresses. The inhibitory effects of NaCl were sever on *G. max* and *T. aestivum* was tolerant. The accumulation of total soluble sugars were more in *T. aestivum* plants. With increasing of cadmium in nutrient solution starch content was decreased in 2 plants. The number of survival *T. aestivum* plants at sever conditions of these 2 stresses aloneness were more than *G. max* plants.

By addition of different concentration of cadmium to solutions with salinity, final responses of *G. max* plants were better than *T. aestivum* plants, the number of *G. max* plants increased then previous conditions. With increasing of different contents of Cd to nutrient solutions with different contents of salinity the inhibitory effects of these stresses on different growth parameters such as RGR, LWCA, RLAGR, ULR, SLA, SDM, RDM, SFM, RFM, LA, were decreased and *G. max* plants were shown better responses to this conditions then *T. aestivum*.

Abbreviations: LA — leaf area; LWCA — Leaf Water content per unit leaf area; RDM -Root Dry matter; RFM — Root Fresh matter; RGR — Relative Growth Rate; RLAGR — Relative leaf area Growth rate; SDM — shoot Dry matter; SFM — shoot Fresh matter; SLA — Specific leaf area; ULR — Unit leaf rate.

EFFECTS OF CADMIUM ON CALCIUM, IRON, ZINC, MAGNESIUM, SODIUM AND POTASSIUM UPTAKE IN SOYBEAN (*GLYCIN MAX*)

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Three day–old etiolated soybean seedlings were grown in Hoagland solution in present of $Cd(NO_3)_2$. $4H_2O$ (0, 1, 5 and 20 ppm Cd^{+2}). Cd uptake and its effects on Ca, Fe, Mg, Zn, Na and K uptake and their transport to different parts of plants were studied. Cd interfere with uptake an transport of these metal ions. The interaction depends on the Cd concentration and nature of ions. With increasing of Cd content in nutrient solution ,the content of Cd in root, stem and leaves of soybean plants were increased but transport to leaves was low. With increasing of external concentration of Zn, Mg and Na in shoots and roots of plants increased. But decreasing of K and Fe contents were shown with increasing of Cd contents in nutrient solution.

EVIDENCE FOR HEAVY METALS INHIBITION OF ALGAL GROWTH IN LAKE PAMVOTIS (GREECE)

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Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystems and they are a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnification in the food-chain.

Although limited amounts of trace metals are essential for biological activity in lakes, they become toxic for aquatic organisms. Biochemical interactions between algae and metals have been studied in marine environments, but fresh water ecosystems have received less attention. Moreover most work has involved laboratory experiments rather than field studies.

In the present study the effect of Cu and Pb on Chlorophyll-a production as a net growth rate of algal biomass index, in Lake Pamvotis (Greece) was investigated. Lake water samples collected from two sampling-stations (S1, S2) were metal treated to 0.05, 0.1 and 5μg/l final concentrations, in three replicates each one, for both Cu and Pb correspondingly. Samples as well as controls were incubated for 48h under simulated in situ conditions of temperature, light level and photoperiod. Upon termination of incubation, Chlorophyll-a was, spectrophotometrically, determined after the acetone extraction of the pigment.

Additional limnological parameters (included temperature, pH, diss.oxygen and nutrients) were measured.

Copper and Lead additions resulted in reduction of algal biomass. Significant differences between the two metals effects were also registered. At hypertrophic station (S_2) algal biomass was supressed by metal treatment even at 0.05 μg/l metal concentration. At the eutrophic station (S_1), Cu produced a supressive effect at low levels, while Pb reduced algal biomass at 5 μg/l.

SENSITIVITY OF *FUSARIUM SOLANI* PLASMA MEMBRANE TO HEAVY METALS AND REACTIVE OXYGEN SPECIES

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The method of electroorientational spectroscopy was used to study disturbances in the barrier properties of the cell plasma membrane (PM) of fungus *Fusarium solani* under the action of heavy metal (HM) ions (Ag^+, Hg^{2+}, Cu^{2+}) and reactive oxygen species (ROS) (hydroxyl radicals) generated in copper- and hydroxycobalamine-ascorbate systems. The following order of studied HMs toxicity (at studied concentrations) for the *Fusarium solani* PM was shown: $Ag^+ > Hg^{2+} > Cu^{2+}$, which correlated with the affinity of these metals to the functional groups of membrane proteins. When the cells were attacked by ROS, the changes in their membrane permeability were observed only for the catalytic pair copper-ascorbate, probably, due to the difficulties for the large complex of hydroxycobalamine to penetrate through the cell wall, that is why in this binary system the formed hydroxyl radicals were inaccessible for PM.

PIGMENT COMPLEX OF PLANTS AS A SUSTAINABILITY INDEX IN TECHNOGENIC CONDITIONS

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The metals make a considerable proportion of lets of industrial enterprises. The metals render negative effect on plants. It is marked, that effect of metals on plants appear in the form of change of morphological and physiological parameters of plants, personally in contents of pigments in leaves.

The pigment complex of plants represents combination of chemical combinations basic of which are the chlorophyll **A** and **B**, and also carotenoides. If pollutants miss then the contents of pigments and their ratio is supported at a definite level, that provides a maximum effective work of the photosynthetic vehicle of plants. However there is diurnal and seasonal dynamics of the pigment contents in plant leaves.

The expediency of realization of the present researches is stipulated by that the plants at stresses are compelled to adapt for new conditions by modification of physiological and biochemical processes. For this purpose the significant amount of energy is necessary, the resource is provided with which one in many respects by activity of the photosynthetic vehicle. Thus, quantity and ratio of pigments in plant leaves can serve criterion of potential stability, and ability fast to change the contents of pigments outgoing from needs of an organism — criterion of adaptive capabilities of a kind for survival in technogenic conditions.

The researches conducted on plants of a balsam poplar (*Populus balsamifera* L.) in conditions of sand culture. After formation leaves and the assemblages of roots plants was treated by aqueous solutions of acetates of metals of sublethal density. Then watched of dynamics of change of the main pigments of a photosynthesis — chlorophyll **A**, **B** and carotenoides.

It was established, that at operation Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} the sum of pigments in balsam poplar leaves is reduced more than on 20% concerning the control. The ratio of pigments changes so, that there is a decrease of a share of chlorophyll A, at increased shares of chlorophyll **B** (for Mg^{2+}, Mn^{2+}) and carotenoides (for Ca^{2+}, Zn^{2+}, Cu^{2+}) in leaves of experimental plants. At operation the ratio of pigments in leaves of control plants was saved at a level: chlorophyll **A** — more than 50 %, chlorophyll **B** — about 30 %, carotenoides — less than 20%.

The correlation between change of quantity indicators of the photosynthesis pigment contents and stability of plants is established. At increase of density of metals in an environment there is a quantitative and functional reallocation of pigments in leaves of a balsam poplar. It was rotined, that due to activity of adaptive gears the stability of an organism is supported at a level of a pigment complex (at a decrease of the contents of the main pigment of a photosynthesis — chlorophyll **A** in leaves there is an increase of auxiliary pigments — chlorophyll **B** and carotenoides) so, that in the environment pollution conditions by metals of plant survive.

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THE INFLUENCE OF ENVIRONMENT POLLUTED WITH SOME METAL IONS ON GENERATIVE SPHERE OF PLANTS (PALYNOTERATICAL DATA FROM THE LENINGRAD ATOMIC POWER STATION AREA)

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The rate of heavy– and radioactive metal ions (except copper and zinc) and benz(a)-pirene in the Leningrad Atomic Power Station (APS) industrial zone is not high, though the quantity of Mn, Co, Ni, As and Pb ions in separate points exceeds normal concentrations (Dzuba et al., 2001). The level of chemical contamination in the town of Sosnovyi Bor is higher than at the APS.

A new statistical palynoteratical method (G.M. Levkovskaya. Palynoteratical Complexes as Indicators of Ecological Stress, Past and Present// Proceedings of 5th European Palaeobotanical and Palynological Conference. Acta Palaeobotanica 2, Krakow, 643-648, 1999) allows to use complexes with domination of morphologically abnormal pollen of different types as indicators of normal, stressed and catastrophic states of generative sphere of single plants and even their societies.

A specific feature of pollen complexes of soil samples from Sosnovyi Bor is high percentage of palynoterates with divergence from the norm in several morphological characters simultaneously which was proved by statistical and SEM data.

This type of palynoteratical complex is a “response” of the plants’ generative sphere on an anrtropogenic stress. It was a result of polluting the area with heavy and radioactive metal ions.

A new method of environmental control could be

worked out as a result of joint research projects of the author of the palynoteratical method (Levkovskaya, 1999; see e-mail for contacts) and specialists on metal ions.

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THE TOLERANCE OF *CALENDULA* FAMILY PLANTS TO INCREASED HEAVY METAL CONTENTS

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Background & Aims: The research of plant stability to the toxiferous factors is urgent in the conditions of an increasing antropogenic environmental pollution. The results of research on this subject relevant to preserve the purity of food chains and consequently to keep people healthy.

Methods: The research was conducted on sixty day old plants of *Calendula arvensis* L. and three kinds of *Calendula officinalis* L.: Kablouna Golden, Caltha, Californian. During the experiment the wet and dry weights of shoots and roots, relative growth rate (RGR), gross-photosynthesis (Pg) and rate of respiratory costs for gross-photosynthesis (R / Pg) were determined.

Results & Conclusions: The experiment led to the conclusion that:

1. The addition of copper salts in the medium caused insignificant of alteration of morpho-physiological parameters for all investigated plants.
2. The reaction of investigated plants to the addition of zinc and cobalt salts depended on the species. The plants of Kablouna Golden were more tolerant in the degree of change of relative growth rate and the ratio of general respiration to growth-photosynthesis, less tolerant the ones of Caltha sort.
3. It was shown that for the more objective and informative characteristics stability of plant at the level of morpho-physiological parameters under the influence salts of heavy metal it is preferable to use relative parameters such as relative growth rate and part of respiratory costs for gross-photosynthesis.

THE INFLUENCE OF HEAVY METALS ON PLANT RADIOSENSITIVITY WITH THE *HORDEUM / TRITICUM* ROOT-CHROMOSOMAL ABERRATION ASSAY

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The disturbances of the chromosome apparatus in cells of seedlings of *Hordeum vulgare* and *Triticum durum* seeds collected from plants growing in the zone of direct action of the trucking-highway complex were studied. Ears were collected along the highway near the town of Pushchino of the Moscow region: experimental samples at a distance of 6 m and control samples, at a distance of 70 m from the border. Dry seeds were irradiated with ^{60}Co γ-rays with a dose of 148 Gy at a dose rate of 33 Gy/min. Radiation damage was estimated by the anaphase assay. Earlier, the mass of a thousand seeds, the size and mass of one ear, the number of seeds in one ear, and the content of heavy metals after ashing of seeds at 450°C were determined. The most pronounced changes were observed in the mass of seeds from plants growing near the highway: in wheat, it decreased 1.34 times and in barley, 1.25 times (Nikolaeva et al., 1985).

The analysis of the chromosome aberrations revealed no differences between the control and experimental groups. However, after exposure of seeds to gg-rays, differences in the sensitivity of chromosomes to radiation were revealed. The yield of structural damage of chromosomes after irradiation with the same dose was substantially higher in plants growing at a distance of 70 m from the highway border compared to plants growing near the highway. The increased radioresistance of seeds from plants growing in the 6-m near-highway zone correlates with the level of heavy metals accumulated in seeds. Presumably, the accumulation of metals is responsible for the increased seed radioresistance. A comparison of radiobiological data with the results of physiological and biochemical and morphological studies indicated that the radiobiological method of analysis of changes in the cell chromosomal apparatus can be utilized for ecological examination.

TECHNETIUM VALENCIES IN PLANTS

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Plants have the ability to accumulate the long lived fission product ^{99}Tc. In this work, an attempt was made to separate and characterize technetium species induced by cereals plants grown on soil containing $Tc^{VII}O_4^-$ nutrient solution. Combination of data obtained with selective extraction (ammonium sulfate, acetone, diethyl ether and 8-hydroxyquinoline in chloroform) and use of Liquid Scintillation and Phosphorimager gave us insight into Tc speciation in cereals.

A phosphorimager-based technique is used to detect and quantify Tc in solution by autoradiography. This technique offers several advantages: a very high sensitivity and minimizing disposal procedures. We used the Phosphorimager technique in conjonction with paper chromatography to quantify different oxidation states of ^{99}Tc in solution. Different radiochemical procedures have been used to prepare reduced Tc IV and V standards from pertechnetate $Tc^{VII}O_4^-$ by $SbCl_2$ and other reducers. After chromatography on Whatmann 3MM in HCl 0.3 M as mobile phase, the associated spots of migration for the different valencies are: $RfTc^{V} = 0.0$, $RfTc^{VII} = 0.7$, $RfTc^{IV} = 0.9$.

Rf values have been measured in different mobile phases (HCl 0.3N, NaCl 0.9%, acetone, acetonitrile/water).

The following classes of Tc species in leaf homogenate were found after a cultivation on earth enriched by pertechnetate : $Tc^{VII}O_4^-$, Tc^{V} -cystein, Tc bound to insoluble cell-wall polysaccharides, Tc bound to proteins, and hydrophilic non-protein Tc species. These results may yield a new insight into the metabolic pathways of Tc in plants.

CONTENT OF SOME METALS (CA, MN, FE, SR) IN *LARIX SUKACZEWII* DYL. ROOT SYSTEM UNDER POLLUTION CONDITION (UFA INDUSTRIAL CENTER)

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The metals have important role in regulation of physiological and biochemical processes of plants. The manganese (Mn) participates in formation of chlorophyll, stimulates photosynthesis and adjusts of oxidation-reduction processes (Vlasuk, 1948; Gerretsen, 1950). The lack of iron (Fe) causes decrease of the chlorophyll contents in leaves (Vlasuk et al., 1974). The calcium (Ca) participates in formation of cell walls and synthesis of albumen substances (Burstrom, 1954; Dvorakovsky, 1983).

The purpose of work was the study of the some metal content (Ca, Mn, Fe, and Sr) in root system of *Larix sukaczewii* Dyl. under petrochemical pollution conditions of the Ufa industrial center (The Preural region, Russia). Object of research was the forest cultures of *L.sukaczewii* (40–50 years old). The metal content was determined by a method of the X-ray-fluorescent analysis on WRA-2 device (Germany). The root sample for tests undertook up to depth 1 m, the metal content was estimated in mg/kg of air-dry substance.

It was established, that under petrochemical pollution conditions the Ca content in *L.sukaczewii* roots makes 3480–22620 mg/kg, Mn — 13–540 mg/kg, and Fe — 3010–9280 mg/kg, in the control ground — 4040–15840 mg/kg, 40–150 mg/kg, and 1030–4040 mg/kg accordingly. In all tests the strontium (traces) present too.

Under pollution conditions in the top soil layers in *L.sukaczewii* roots the increase of the Ca content (in 2–4 times), Mn (in 1.5–2.5 times), and Fe (in 3.5 times) was marked.

The increase of the metal content in *L.sukaczewii* roots under pollution conditions can be considered as an adaptive reaction of the root system and tree (as a whole) growing in industrial pollution conditions directed on a survival of *L.sukaczewii* under extremely conditions of technogenesis.

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