1. PLENARY LECTURES

L01 FUNCTIONAL DAIRY PRODUCTS – FROM TRADITIONAL TO MODERN BIOTECHNOLOGY

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Introduction

There are many aspects of functional food concept and as a consequence this term has no exact definition. It is usually described as "food which promotes health beyond providing basic nutrition".

In order to establish a scientific basis to the new areas of functional foods, the European Functional Food Science Programme (FUFOSE) was set up. The following definition was proposed[1]: »A food can be regarded as "functional" if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease«. Some additional conditions were stressed as well: »Functional foods must remain foods, and they must demonstrate their effects in amounts that can normally be expected to be consumed in a diet. According to this definition a functional food can be[2]:

- A natural food in which one of the components has been naturally enhanced through special growing conditions.
- A food to which a component has been added to provide benefits
- A food from which a component has been removed so that the food has less adverse health effects.
- A food in which the nature of one or more components has been chemically modified in order to improve health.
- A food in which the bioavailability of one or more components has been increased to provide greater absorption of a beneficial component.
- Any combination of the preceding possibilities.

Dairy Products in the Light of Functional Food

Milk and dairy products have long been seen as a »healthy food« and an essential component of a diet. Yoghurt is perhaps the most popular of all fermented milks. The belief in its beneficial influence on human health has existed in many civilizations over a long period of time. In Ayurveda, one of the oldest medical sciences that date from around 2500 BC, the consumption of yoghurt has been recommended for the maintenance of overall well being[3]. In 76 BC the Roman historian Plinius recommended the administration of fermented milk for treating the gastrointestinal (GIT) disturbances[4]. Therefore, fermented milk could be regarded as nature's original functional food. However, not earlier than nearly 2000 years later man started to recognize the agents which convert milk to the sour milk - the Lactic Acid Bacteria (LAB).

Whey, a co-product of cheese-making, was discovered about 3000 years ago. There are historical references about the use of whey as aphrodisiac and for medical purposes, including sepsis, wounds' healing, and 'stomach disease'[5,6]. Nowadays, sound scientific substantiation of the nutritional and biological qualities of whey components, notably the proteins, is increasing and whey-based ingredients have become prominent representatives of the functional foods.

Today we know that milk contains an array of bioactive peptides and is rich in antioxidants, highly absorbable calcium, conjugated linoleic acid (CLA) and other biologically active components. Nutritional and genetic interventions to alter the milk composition for specific health and/or processing opportunities are gaining importance in dairy biotechnology. Less fat in milk, altered fatty acid (FA) profiles with less saturated FA and more healthy FA such as CLA, improved amino acid profiles, less lactose, and absence of β-lactoglobulin are some examples of milk »designing« [7,8]. Furthermore, a wide range of products can be obtained from this nourishing liquid.

"The variety of functional foods that can be developed is limited by our imagination" wrote Spence[9] who grouped functional foods in four types (Table I). Although dairy products can be found in each type, it has to be stressed that we may use the term "functional" only for products with proven health benefits.

Table I
Different types of functional foods

Type	Characteristics	Example of dairy products
Fortified products	Increased content of existing nutrients	Products fortified with Ca, vitamins or antioxidants (Q10)
Enriched products	New nutrients or components added (not normally found in particular food	Products enriched with pro-, pre-biotics
Altered products	Existing components replaced or chemically modified	Products with fat replacers (fat free) Hydrolysed milk
Enhanced commodities	Changes in the raw commodities that have altered nutrient composition	Milk with altered FA profile (less saturated FA, more omega-FA and CLA. Milk with higher level of melatonin.

Bioactive Components of Functional Dairy Products

Milk is a natural source of a variety of beneficial nutrients and biologically-active compounds with potential impacts on the human health. Designing and developing functionality

in dairy products simply means modifying and/or enriching the healthy nature of the original base. In addition, progress in food processing and biotechnology offers new possibilities in creating foods with special characteristics and functionality. Among the most prominent representatives of functional dairy products are certainly fermented products.

The health promoting effects of the fermented dairy foods may be related to the biological activity of the lactic acid bacteria (LAB) used in the production of these products.

In theory, LAB can exert beneficial effects through two mechanisms: direct effects of the live microbial cells or indirect effects via biogenics. The special type of bacteria associated with "healthy" gastro intestinal tract (GIT) has been classified as "probiotic" bacteria. By FAO/WHO[10] definition, probiotics are live microorganisms which, when ingested in adequate amounts, deliver measurable physiological benefits, which are usually strain specific. Biogenics are defined as food components derived from microbial activity which provide health benefits without involving intestinal microflora[11]. According to this definition the biogenics of fermented milk are the substances such as lactic acid, butyric acid, bioactive peptides, β-galactosidase, and exopolysaccharides produced by LAB during fermentation.

Table II
Health benefits of probiotic cultures

Health benefit	Mechanisms
Prevention/treatment of GIT infections, diarrhea	Antimicrobial activity (organic acids, hydrogen peroxide, bacteriocins) Depletion and/or competition for substrates Occupation specific binding-sites Reduction of luminal pH
Improvement in lactose metabolism	Production of β-D-galactosidase
Antimutagenic properties	Binding of mutagens Reduction of faecal enzymatic activity (β-glucuronidase, azoreductaze, nitroreductaze) Production of butyric acid
Anticarcinogenic activity	Reduction of faecal enzymatic activity (β-glucuronidase, azoreductaze, nitroreductaze) Production of short chain fatty acid Normalization of intestinal permeability Strengthening of intestinal barrier Boosting of immune system
Reduction in serum cholesterol	De-conjugation of bile salts Taken up cholesterol during growth
Prevention/treatment of *Helicobacter pylori* infection	Reduction of bacterial load Inhibition of growth of *H. pylori*
Stimulation of immune system	Directly or indirectly stimulation by changing the composition or activity of intestinal microflora

Functional Cultures

In the dairy biotechnology, the LAB are the most important bacteria that lead all fermentations. LAB inhabit two main ecological niches: the mucous surfaces of man and animals, and fermented foods. The acidification and enzymatic processes accompanying the growth of LAB impart the key flavour, texture, and preservative qualities to a variety of fermented foods. The LAB, notably lactobacilli, which occupy important niches such as the gastrointestinal tracts (GIT) are considered to contribute a number of probiotic benefits to general health and well being[12] (Table II).

The search for a starter culture has until recently been relying on the screening of a large number of isolates in small-scale food fermentations. Molecular biology has fostered the development of genetically based selection tools allowing us to specifically target the individual genes and metabolic pathways responsible for desired performance parameters of a starter culture. Specific targeting makes screening by high throughput methods possible, and it opens the possibility to use mutant selection and genetic engineering to construct novel starters. The engineering of recombinant starter cultures by the aid of food grade techniques can now be done on a safety level matching or even exceeding the safety level of "natural" screening and selection[13,14,15].

Examples of novel strains obtained by the use of modern techniques are:

- a spontaneous roseoflavin-resistant mutant derived from *Propionibacterium freudenreichii* B374 that shows increased riboflavin production and was used as adjunct culture for production of yoghurt with improved nutritional value[16],
- a non-D-lactate-producing variant of probiotic *Lactobacillus johnsonii* La1, convenient for the patients suffering from short bowel syndrome and intestinal failures[13].

Probiotic cultures production technologies should provide in a cost effective manner high quality, safe end-products with long shelf life. Working with living microbes is demanding since viability losses occur easily if microbial cultures are exposed to stressful conditions. Technological robustness can be improved by activating the stress genes of bacterial cells during processing by suitable sub- lethal treatments, or by genetically modifying the strain to make it more robust[17].

Biogenics

During the milk fermentation by LAB the main chemical change is fermentation of lactose into lactic acid. The functional properties of lactic acid are numerous: it reduces pH, influences the physical properties of casein and promotes digestibility, improves the utilisation of calcium and other minerals, and inhibits the growth of potentially harmful bacteria.

The liberation of short- and medium-chain free fatty acids during fermentation and in the stomach after milk fat ingestion, contributes to a lowering of the pH which facilitate digestion of proteins and inhibit the pathogenic bacteria. In addition, the fatty acids and monoglycerides with chain lengths varying from 8 to 12 carbon atoms display antibacterial and antiviral activity[18]. Recent studies confirmed the potential of these FAs to reduce body weight, particularly body fat[19]. Butyric acid formed during fermentation in milk or in the colon, affects intestinal cell differentiation, apoptosis and cell growth control. It is also known that butyrate displays anti-colorectal cancer properties, probably by enhancing apoptosis of mutant colonic cells[20].

Much attention has been directed toward conjugated linoleic acid (CLA) since the discovery of its anticarcinogenic properties. Strong evidence from animal trials supports an influence of CLA on body composition, i.e. lowering of body weight and fat mass and a relative increase in the lean body mass. Results from human trials do not support a weight loss-inducing effect of CLA but indicate a body fat lowering effect associated with an increase in the lean body mass[21]. Cows' milk fat is the richest natural source of CLA and its quantity can be raised by cow's nutrition. Possibilities to increase the CLA content of dairy products with microbial cultures have been studied by many authors however, their contribution in increasing CLA in dairy products seems to be minor[22]. Enrichment of dairy products with encapsulated CLA seems more perspective way for producing CLA rich dairy products[23].

Many LABs are known to produce exocellular polysaccharides or EPSs. Their precise function still remains unclear, although it is considered to be related to cell adhesion and cell protection in different ecosystems. LAB that produce EPSs play an important role in dairy industry because of their contribution to the texture, mouthfeel and stability of fermented milk. Besides their technological functionality, certain EPSs produced by LAB are also claimed to have beneficial physiological effects. It is speculated that the increased viscosity caused by EPSs may increase the residence time of ingested fermented milk in the GIT and in this way also enable transient colonisation of probiotic bacteria. Because of the special composition and low degradability, EPSs can act as prebiotics and thus beneficially affect the host by selectively stimulating the growth and activity of beneficial intestinal bacteria in the colon. Suggested health benefits of some EPSs are antitumor, antiulcer, immunomodulating and cholesterol-lowering activities[24,25,26].

Probably the most important as well as thoroughly studied biogenics in dairy products are whey proteins and bioactive peptides. Whey proteins have been implicated in a variety of nutritional and physiological effects, including physical performance, recovery after exercise, and prevention of muscular atrophy, satiety and weight management, cardiovascular health, anti-cancer effects, wound care and repair, management of infections, infant nutrition and healthy aging[6].

Milk proteins are rich source of bioactive peptides. Milk proteins-derived bioactive peptides are inactive as long as are a part of parent protein and can be released by enzymatic proteolysis. Once they are liberated, bioactive peptides may act in the body as regulatory compounds (Table III). Many of bioactive peptides demonstrate multifunctional activity. For example, some regions in the β-casein contain overlapping peptide sequences which exert different biological effects. These regions are called "strategic zones" and are partially protected from proteolytic breakdown[27,28,29].

Table III
Physiological functionality of milk-derived bioactive peptides[29]

Activity	Target system
Antihypertensive Antioxidative Antithrombotic Hypocholesterolemic	CARDIOVASCULAR SYSTEM
Opioid • agonist activity • antagonist activity	NERVOUS SYSTEM
Mineral-binding Anti-appetizing Antimicrobial	GASTROINTESTINAL SYSTEM
Antimicrobial Immunomodulatory Cytomodulatory	IMMUNE SYSTEM

Functional Dairy Products and Health

Hypertension

High blood pressure is one of the most important risk factors of coronary heart disease. Studies have shown that during fermentation of milk ACE-inhibitory peptides arise from caseins[29]. The best known ACE-inhibitory peptides are Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP). At present, at least two fermented sour-milk products containing the ACE-inhibitory three-peptides VPP and IPP have been launched commercially. The Japanese product "Calpis" fermented by *L. helveticus* and *Saccharomyces cerevisiae* contains two ACE-inhibitory peptides derived from β-casein f84-86 (VPP) and f74-76 (IPP) and one from κ-casein f108-110 (IPP)[30]. The Finnish product "Evolus" contains the same three-peptides produced by *L. helveticus* LBK-16H strain. Both of these fermented drinks have proven effects in the reduction of blood pressure in hypertensive human subjects[29].

Gastrointestinal Health and General Well-Being

The intestinal microflora is important for maintenance of host health, providing energy, nutrients and protection against pathogens. Although the colonic microflora is relatively stable throughout life, age-related changes in the GIT as well as changes in diet and host immune system reactivity affect population composition[31]. Since the LAB are essential in

the maintenance of intestinal homeostasis and consequently in general human health, functional fermented dairy products have been proposed as healthy foods to control intestinal disorders. The results of an extensive research work have confirmed their beneficial effect in the case of lactose intolerance, GIT infections, viral diarrhoea, antibiotic-associated diarrhoea, in prevention and treatment of traveller's diarrhoea and *Heliobacter pylori* gastritis. Less consistent results have been obtained studying the effects of functional dairy products on the side effects of radiotherapy, constipation and in cancer prevention[32].

Lactose Intolerance

The most documented beneficial effect of fermented milks, especially yoghurt, on the intestinal function is observed in the case of lactose intolerance[33,34]. Lactose intolerance is caused by a deficiency in the enzyme beta-galactosidase, resulting in the inability to digest the disaccharide, lactose. The symptoms of lactose intolerance are diarrhoea, flatulence and abdominal pain after consumption of milk. The mechanisms involved in the beneficial effects of LAB and fermented milks in case of lactose intolerance are: lower lactose concentration in the fermented milk, high lactase activity of LAB used as starter cultures and active lactase present in fermented milk or entering the small intestine within the bacterial cells able to survive gastric and bile conditions.

Diarrhea

One of the main applications of probiotics has been the treatment and prevention of antibiotic-associated diarrhoea, which is often caused by occurrence of *Clostridium difficile* after an antibiotic treatment. The application of probiotics in the clinical setting significantly reduced antibiotic-associated diarrhoea by 52 %, reduced the risk of travellers' diarrhoea by 8 % and that of acute diarrhoea of diverse causes by 34 %. Moreover, the associated risk of acute diarrhoea among children or adults was reduced by 57 % and 26 %, respectively[35]. Several possible mechanisms by which fermented dairy foods containing probiotics or cultures containing milks reduce the duration of diarrhoea, are competitive exclusion, translocation/barrier effect and improved immune response[34].

Conclusions

Development of functional dairy products requires various groups of experts including medical experts, nutritionists, microbiologists and food technologists. By using an individual's unique genetic makeup and nutritional requirements, consumers may have in the future a greater possibility to choose between different functional dairy food products and to consequently reduce their risk of disease.

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L02 ALKALI-ACTIVATED ALUMINOSILICATES: PAST, PRESENT AND FUTURE

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Introduction

Beginning from 1957, a scientific school in Kiev, USSR, headed by Professor Victor Glukhovsky has been developing a new direction in the binding materials, the background of which was the discovery of binding properties of the alkali metals. The alkali metals compounds act not only as activators of hardening but are responsible for the formation of main structural elements of the alkali-activated cements – zeolite-like compounds of different types.

Just these hydration products, analogues to natural zeolites of the $Na_2O(K_2O)\cdot Al_2O_3\cdot(2\text{-}4)SiO_2\cdot 2H_2O$ type, have been discovered in ancient concretes (Ancient Greece, Ancient Rome, Egypt, Syria). Durability of the ancient concretes and similarity of their structure with that of the alkali- activated cement concretes allowed to predict their high durability. Effectiveness and high performance properties of the alkali-activated cement concretes are supported by over 50-year experience of service of the structures made from them.

Theoretical Background

Less than a century ago just an idea of the presence of free alkalis in a cement composition was treated by cement people as absurd one and this was a basic postulate of fundamentals of exhibiting hydraulic by mineral systems. The alkali metal compounds were excluded from the traditional hydraulic cements because of their high solubility.

At the same time, the studies held to reveal the reasons for excellent durability of ancient cements in combination with the data collected on stability and composition of natural mineral formations testified that this postulate was not correct.

In 1957 the scientist from the Soviet Union Victor Glukhovsky has discovered that compounds of alkali metals (Li, Na, K, Rb, Cs) – the elements of the first group of the Periodic Table, exhibit hydraulic binding properties similar to compounds of the alkali earth metals (Mg, Ca, Sr, Ba)- the elements of the second group.

Thus, an idea of creation of the cementitious systems was transformed as the following:

$$\left.\begin{matrix} CaO - SiO_2 \\ CaO - Al_2O_3 \end{matrix}\right\} + H_2O \rightarrow \begin{matrix} CaO - SiO_2 - H_2O \\ CaO - Al_2O_3 - H_2O \end{matrix}$$

"Old scheme" (ordinary Portland cement, high-alumina cement)

$$\left.\begin{matrix} Al_2O_3 - SiO_2 \\ RO - Al_2O_3 - SiO_2 \end{matrix}\right\} + R_2O + H_2O \rightarrow \begin{matrix} R_2O - Al_2O_3 - SiO_2 - H_2O \\ RO - R_2O - Al_2O_3 - SiO_2 - H_2O \end{matrix}$$

"New scheme" (alkali- activated cement)
where R – Na, K, Li, Rb, Cs

The idea itself of using these systems as cementitious ones was based, first of all, on geological data that sodium-potassium- calcium aluminosilicate compounds, which are known to have the higher stability and resistance to atmospheric reagents, are present in the Earth. Secondly, this idea was based on the results of experimental studies, which proved that alkali hydroxides and salts of alkali metals came into interaction with clay minerals, aluminosilicate glasses and crystalline substances of natural and artificial origin with the formation of water resistant alkaline and alkaline- alkali-earth aluminosilicate hydration products analogous to natural minerals of the zeolite and mica types.

Terminology

The established possibility of modelling the processes taking place in the Earth's crust based on an interaction between the decay products of rock-forming minerals: clays and alkalis, followed by synthesis of these minerals, suggested using natural soils as starting materials for producing a binder. This explains why the binders/cementitious materials/ developed as long ago as in 1957 have been called "soil cements" and the concretes "soil silicates"[1].

The alkaline and alkali-earth hydroaluminosilicates analogous to natural minerals (hydronepheline, analcime, natrolite, thompsonite, hydrosodalite, etc.) are formed in the soil cement stone during the process of hardening along with calcium hydrosilicates and carbonates.

The soil silicates contain alkalis in large amounts (1–20 % wt. calculated on R_2O). Just the alkaline oxides are the components which determine their binding properties. The alkali earth oxides are either absent in them (fly ashes, cakes, clays, field spur minerals) or are present in the aluminosilicate component (cakes, fly ashes, slags) or may be introduced from the outside as the components of the traditional binding materials (lime, Roman-, Portland-, slag Portland or high alumina cements).

In 1973, Professor J. Davidovits was granted his first patents for the geopolymers[2]. The technology for manufacturing these cements included the following steps: mixing kaolinite, lime stone, dolomite; burning of a mix and introduction of alkaline compound solutions. During these processes, the kaolinite converts to the metakaolinite ($Al_2O_3\cdot 2SiO_2$), gaining the pozzolanic properties, while calcium and magnesium carbonates form calcium and magnesium oxides. Being added to a cement mix, silica or soda or its mixture with potash incorporated with a mixing water produce sodium and potassium hydroxides. The last initiates a chemical reaction with

polysilicate and aluminosilicate oxides with the formation in a composition of the hydration products, represented by analcime and hydrosodalite. Some of these products are known as commercial products (trade names: Pyrament, Geopolycem, Geopolymite, etc.). The cements of this type are known in the art under a general name "geopolymers".

The alkali-activated cements are known among cement people also under other names: alkali-activated cements[3] SKJ-binder[4], F-cement[5], gypsum-free Portland cement[6], geocements[7].

Classification

The classification proposed by[8] is based on the characteristic features of the products of hydration and hardening of the alkali-activated cements, the "edge" variants of which may be represented by the compounds of two types: the alkaline hydrosluminosilicates of the system $R_2O – Al_2O_3 – SiO_2 – H_2O$ and the earth hydrosilicates.

A variety of blended alkaline-alkali-earth hydroaluminosilicates may exist within these "edge" variants. A phase composition of the hydration products of the cement stone is determined by a kind of the initial raw material (Table I).

Table I
Mineralogical composition of the cement hydration products vs type of initial aluminosilicate component

Cement type	Initial soild phase	Alkali content R_2O [%]	Hydration product	
OPC	OPC clincker	<0.6	0%	100%
Alkali-activated OPC	OPC clinker + R_2O	1–5	R_2O-Al_2O_3-SiO_2-H_2O	RO-SiO_2-H_2O
Alkali-activated blended OPC	OPC clinker + additive (slag, ash, basalt) + Me_2O	2–5		
Slag alkali-activated cement	Metallurgical slag + R_2O	4–8		
Ash alkali-activated cement	Ash – product of coal combustion	5–10		
Geocement	Clay + R_2O	10–20	100%	0%

Principles of Compositional Build-up of the Alkali-Activated Cements

The following postulates have been laid down in their creation:

- alkalis act not only as activators but as structure- forming elements included into the formed phases as well;
- the formed phases (of hydration products) are characteristic of the presence of new formations of the $R_2O–Al_2O_3 – SiO_2 – H_2O$ and $R_2O – CaO – Al_2O_3 – SiO_2 – H_2O$ types;
- the quantities of alkalis to be introduced are caused by a necessity to meet a stoichiometric composition /stoichiometry requirement/ of the alkaline and alkaline-alkali-earth hydroaluminosilicates analogous to natural zeolites.

In compliance with these principles the alkali content in the cement will be determined by an Al_2O_3 content of the aluminosilicate component (Fig. 1.).

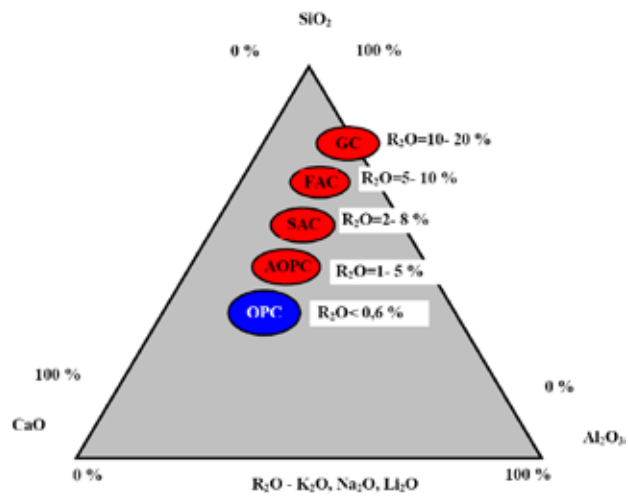

Fig. 1. **Comparative chemical analysis of different types of OPC and alkali-activated cements. Abbreviations: OPC – ordinary Portland cement; AOPC – alkali-activated ordinary Portland cement; SAC – slag alkali-activated cement; FAC – ash alkali-activated cement; GC – geocement**

The introduction into a cement composition of the alkali metal compounds in much larger quantities than was permitted in compliance with the principles of compositional build-up of the traditional cements based on calcium and magnesium compounds suggested to consider that the alkali metal compounds not only act as activators of hardening but as self-functioning components of the binding system $Me_2O – MeO – Me_2O_3 – SiO_2 – H_2O$, the main structure-forming products of which are low-basic calcium hydrosilicates and zeolite-like products. A low basicity of the hydration products is caused by specific features of the structure-forming processes taking place in the slag alkali- activated cements, namely: a hydrolytic destruction of the solid phase of the low-basic phases is caused, first of all, by the break of the covalent

bonds Si – O – Si, Me^{3+}– O – Me^{3+}, Si – O – Me^{3+} according to a scheme ≡ Si – O – Si ≡ ↔ [≡ Si – O – Si ≡]– ↔ ≡ Si – OH + ≡ Si – O – with protonization of the ion Me^{2+}– O bonds taking place in parallel, as it is known to happen in the high-basic systems[9].

An alkaline cation that provides the flow of the hydrolytic destruction of the low-active low-basic phases, acts at early stages of structure formation as a catalyst of destruction. Then, as soon as the condensation processes evolve, it takes an active part as a co-partner of the Ca^{2+} and Mg^{2+} in the structure formation processes. This facilitates its modification due to the formation of the alkaline and alkaline-alkali-earth hydroaluminosilicates that are morphologically homogeneous to low-basic calcium-magnesium hydrosilicate phases.

At early stages of hydration and hardening (for example, of the slag alkali-activated cements), the structure formation is caused, chiefly, by the formation and crystallization of the low- basic hydrosilicates and hydrogarnets. The alkaline and alkali-earth hydro-aluminosilicates, as a result of their slower crystallization, occur at the later stages. Being formed, chiefly, in the pore space, they fill it and promote strong crystallization contacts with primary phases to occur, as well as initiate the formation of more homogeneous and dense structure.

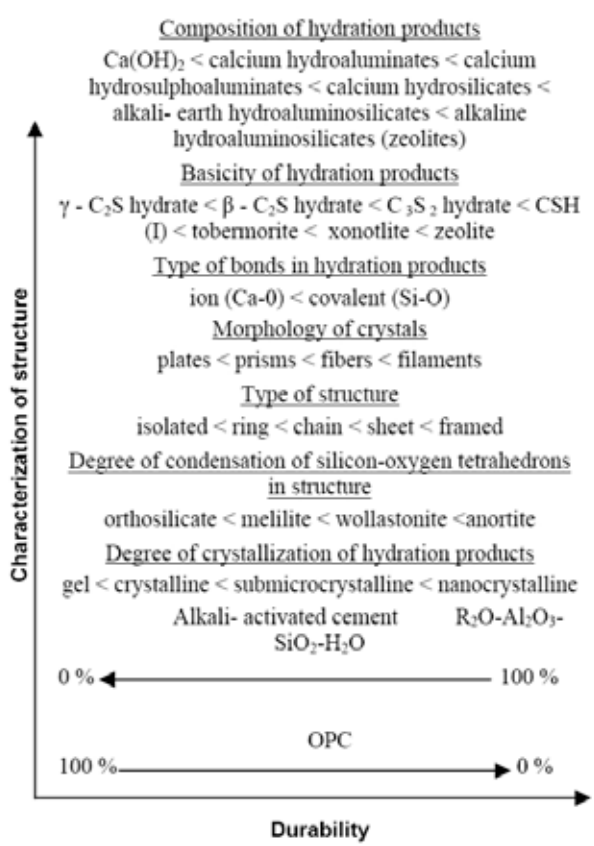

Fig. 2. **Dependence between phase compositions of the hydration products, their structure and durability of the cement stone**

Besides, high pH-values of the medium at which the hydration process takes place block a transfer of the Ca-ions into the solution, thus explaining the absence of $Ca(OH)_2$ and the fact that the resulted calcium hydrosilicate has, as a rule, a basicity over 1.

Table II
Comparative data on solubility of new formations of the slag alkali-activated and portland cement stones.

Cement type	New formations		Solubility
	Mineral	Stoichiometric formula	[kg m^{-3}]
Slag alkaline cement	CSH(B)	$5CaO\ SiO_2\ nH_2O$	0.05
	Xonotlite	$6CaO\ 6SiO_2\ H_2O$	0.035
	Riversideite	$5CaO\ 6SiO_2\ 3H_2O$	0.05
	Plombierite	$5CaO\ 6SiO_2\ 10.5H_2O$	0.05
	Gyrolite	$2CaO\ 3SiO_2\ 2.5H_2O$	0.051
	Calcite	$CaCO_3$	0.014
	Hydrogarnet	$3CaO\ Al_2O_3\ 1.5SiO_3\ 3H_2O$	0.02
	Na-Ca hydrosilicate	$(Na,Ca)\ SiO_4\ nH_2O$	0.050
	Thomsonite	$(Na,Ca)\ Si_2O_3\ Al_2O_3\ 6H_2O$	0.05
	Hydronepheline	$Na_2O\ Al_2O_3\ 2SiO_2\ 2H_2O$	0.02
	Natrolite	$Na_2O\ Al_2O_3\ 3SiO_2\ 2H_2O$	0.020
	Analcime	$Na_2O\ Al_2O_3\ 4SiO_2\ 2H_2O$	0.02
	Calcium hydroxide	$Ca(OH)_2$	1.3
Portland cement	C_2SH_2	$2CaO\ SiO_2\ nH_2O$	1.4
	CSH(B)	$5CaO\ 6SiO_2\ nH_2O$	0.05
	Tetra calcium hydroaluminate	$4CaO\ Al_2O_3\ 13H_2O$	1.08
	Tricalcium hydroaluminate	$3CaO\ Al_2O_3\ 6H_2O$	0.56
	Hydrosulpho-aluminate	$3CaO\ Al_2O_3\ 3CaSO_4\ 31H_2O$	high

Durability

Specific features of the mechanism of hydration and hardening of the alkali-activated cement determine formation of the more effective microstructure of the cement stone at different levels as compared with the Portland cement stone (Fig. 2.). This is also clearly seen from comparison of the data on solubility of new hydration products of the alkali-activated and Portland cement stone (Table II).

Industrial Uses

The experience from the small and large scale industrial uses of the alkali-activated cements gained starting the 1960s in construction (hydropower engineering, road, agricultural, industrial, civil engineering, mining, etc.) gave proofs to high performance properties of the concretes on them. The use of the alkali-activated cement-based materials was found to be especially effective one for specially intended use in many fields besides construction[11].

Below are given some fields of the manufacture and use of the alkali-activated aluminosilicates (Table III).

Table III
Some examples of practical uses of the alkali- activated aluminosilicates

2007		Heavy-duty road pavements, access roads and storage sites of chemical plants					
2000	Inorganic adhesives and glues, protective coatings against action of corrosive environments and high temperatures						
1990				Articles and structures from acid resistant concretes	High-rice building from precast and cast-*in-situ* concrete		Compounds for radioactive waste immobilization Bodies of precise machine tools
1985				Floors, landings from cast-*in-situ* concretes	Blocks for buildings, garages, storage houses, etc.		Dies, moulds
1980				Articles and structures from hest resistant concrete	Floor slabs, foundation wall blocks, foundation blocks, piles	Oil well mortars and grouts	Linings of MD-pumps for aluminium melts
1975		Road bases from strengthened soils					
1970	Tubings of anti-slide systems	Pavements from cast-*in-situ* concrete and precast reinforced concrete slabs	Pasture sites, storage sites for fertilizers, silo pits from cast-*in-situ* and precast concrete	Foundation blocks, floor slabs, columns, beams, foundation wall blocks, elements of cleaning-up systems		Reinforced pit props, sleepers	
1960	Sea breakwaters, elements of irrigation systems from cast-*in-situ* and precast pre-stressed concrete	Pedestrian way slabs, edges of pavement, landing field slabs					
	Hydraulic	**Road**	**Agricultural**	**Industrial**	**Residential**	**Mining**	
				Constructional engineering			**Non-constructional engineering**

Over 45 years passed since the alkali-activated cements appeared in the field and their efficiency and potential have been proved by extensive researches held not only in the Soviet Union but in many countries over the world: Poland, Finland, The Netherlands, Germany, Czech Republic, Romania, Slovak Republic, Bulgaria, Japan, China, USA, Canada, India, Brazil, Spain, the UK. The experience collected for this period of commercial- scale manufacture and use of structures and articles made from slag alkali-activated cement concrete in various fields of construction testifies to their higher service properties as compared with those of Portland cement. These materials were found to be the most highly effective ones when used in extremely severe conditions as well as in non-civil engineering fields (Fig. 3.).

More than 50 years practical experience of the Kiev school is connected with a TECHNOLOGY under which the alkaline activator was introduced into a concrete in a form of aqueous solution, namely:

- "Concrete" – an aluminosilicate component, an alkaline activator and modifying additives are introduced in a dry form or in a form of a solution during mixing concrete ingredients similar to conventional concrete technology.
- "Cement" – an aluminosilicate component, an alkaline activator and modifying additives are interground for further use in concrete under traditional OPC based concrete technology (Fig. 4.).

Standard Test Procedures

Determination of mechanical and physical properties of alkali- activated cements is carried out according to DSTU B V 2.7-24-95 and ASTM C 109/C 109M. According to these standards a water to cement ratio (W/C) is chosen in order to provide a flow value (cone measurement) = 106–115 mm.

In case of using these test methods according to EN standards (EN 196-1, 196-3, and 196-6, under which the

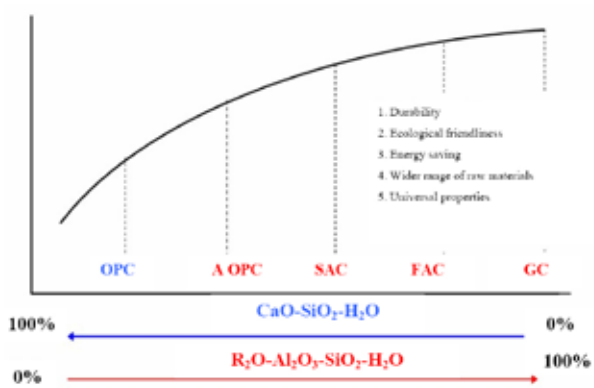

Fig. 3. **Benefits of the alkali-activated cements as compared to Portland cements**

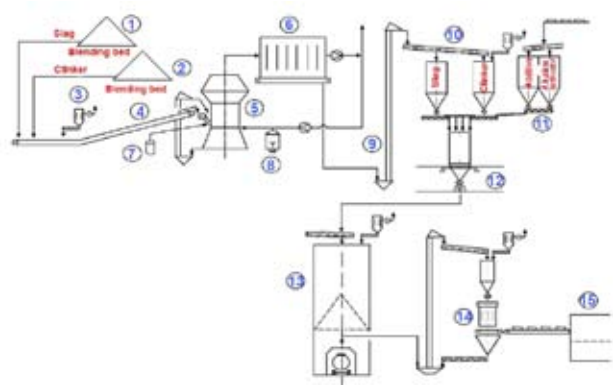

Fig. 4. **"Cement" technology of slag alkali-activated cement manufacture. 1 – blending bed for slag, 2 – blending bed for clinker, 3 – dust collector, 4 – belt conveyer, 5 – vertical roller mill, 6 – fabric filter, 7 – metering equipment for water repellent, 8 – heat generator for drying, 9 – elevator, 10 – silos for cement constituents, 11 – bunker for additives and alkaline activator, 12 – mixer for dry cement constituents, 13 – ready product storage silo, 14 – packer for bags, 15 – palletizer agent**

W/C is restricted to a value of 0.5) the following amendments should be introduced: in determination of flowability of the cement/sand mortar the alkaline solution/solid constituents or water/solid constituents ratio should be chosen experimentally in such a way that to provide the flow values between 160–180 mm.

Conclusions

At the moment Ukraine has all normative documentation required for a large-scale application of the alkali-activated cements and materials.

In order to bring this technology into large-scale worldwide application, the RILEM Technical Committee organized in 2007 "Alkali activated materials" should fulfil the following tasks:

To collect and summarize the experience on:

- raw materials
- cements
- concretes
- structures
- production
- test procedures
- durability
- field of application

To develop basic recommendations "Preparation of a performance-based specification for cast-in-place alkali-activated cements and concretes".

The results of this work will allow to develop and approve the national standards for the alkali-activated materials.

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L03 ORGANIC ANTHROPOGENIC CONTAMINANTS IN RIVER SYSTEMS – AN OVERVIEW ON COMPLEMENTARY ENVIRONMENTAL APPROACHES

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Introduction

Rivers are sensitive but also complex ecosystems hosting a wide spectrum of organisms. Anthropogenic activities and the resulting discharge of contaminants have resulted in increasing river pollution over decades. Especially, in industrialized, densely populated or agricultural strongly affected regions the natural riverine organic matter is superimposed by huge anthropogenic emissions.

Organic contaminants discharged to the aquatic environment exhibit a high diversity with respect to their molecular structures and the resulting physico-chemical properties. Although the chemical analysis of anthropogenic contamination in river systems is still an important feature, especially with respect to the identification and structure elucidation of novel contaminants, the focus of environmental studies has changed. In recent times the research on the environmental behaviour of organic pollutants became more and more important in order to assess their risk as a result of their emission to natural systems. Consequently, the knowledge not only about the occurrence and amount of anthropogenic contaminants but also about bioavailability, their exchange within different compartments, as well as on molecular aspects of degradation or metabolism processes increases significantly. Further on, environmental studies on the anthropogenic pollution of aquatic systems do not remain on the analysis of pre-selected substances. Considering the currently use of over 1,000,000 man-made chemicals in the EU it has to be assumed, that analytical investigations on environmental systems solely focussed on a few pollutants still do not depict the 'real' pollution level. Therefore, the number of more extensive and detailed investigations considering a wide spectra of relevant organic pollutants increases slowly. Such investigations reveal a more comprehensive view on the state of pollution of a natural system[1]. For all the described purposes, a complementary application of different analytical strategies and methods have to be applied to reveal a better understanding of environmental processes affecting the riverine pollution by organic substances. Examples of such approaches are presented following.

River Water

Within river systems two quite differing compartments exist, the particulate matter as well as the water phase, representing a hydrophilic and a more lipophilic phase, respectively. Most of the organic substances accumulate either in the water phase or in the sediments depending on their polarity and the resulting hydro- or liphophilicity. This partition also determines the principal transport processes and, consequently, the distribution of the organic contaminants. The water phase of riverine systems is characterized by more polar and frequently less stable contaminants.

Non-Target Screening

A huge proportion of riverine contamination is caused by low-molecular weight organic compounds, like pesticides plasticizers, pharmaceuticals, personal care products, technical additives etc. Some of them, like PCB or PAH have already been investigated thoroughly and, consequently, their behaviour in aqueous systems is very well described. Since analyses on organic substances in river water traditionally focused on selected pollutants, in particular on common priority pollutants which are monitored routinely, the occurrence of further contaminants, e.g. pharmaceuticals, personal care products or chelating agents has received increasing attention within the last decade. Screening analyses revealed recently an enormous diversity of low-molecular weight organic contaminants in wastewater effluents and river water.[2–5]. Since many of these substances have been rarely noticed so far, it will be an important task for the future to study their occurrence and fate in natural environments.

An outstanding example for an early screening study dated back to the 70'ies. Sheldon and Hites published detailed GC-MS based analyses applied to water samples from the Delaware river[6]. This study presents more than 100 organic river water constituents. A similar approach was used by Franke et al.[2] in order to investigate the organic pollution of the Elbe river. Contributions of several tributaries were characterized by indicative organic compounds derived from industrial, municipal or agricultural sources. Additionally screening analyses were applied to sewage effluents as a major anthropogenic contribution to the riverine environment[3]. All these approaches revealed a high number of organic contaminants and lead to the structure elucidation and identification of numerous still unknown or unreported organic contaminants.

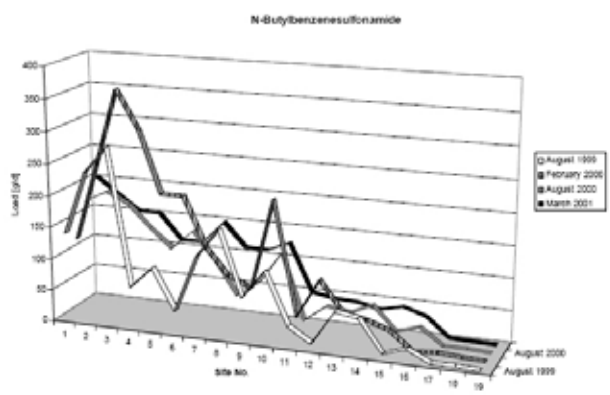

Fig. 1. **NBBS in the longitudinal profile of the Lippe river (according to ref.[7])**

Therefore, it should be a main issue of environmental studies to provide a comprehensive view on the state of pollution of river water, in particular with respect to lipophilic low molecular weight organic contaminants. Such non-target-screening analyses has been performed only rarely in the past.

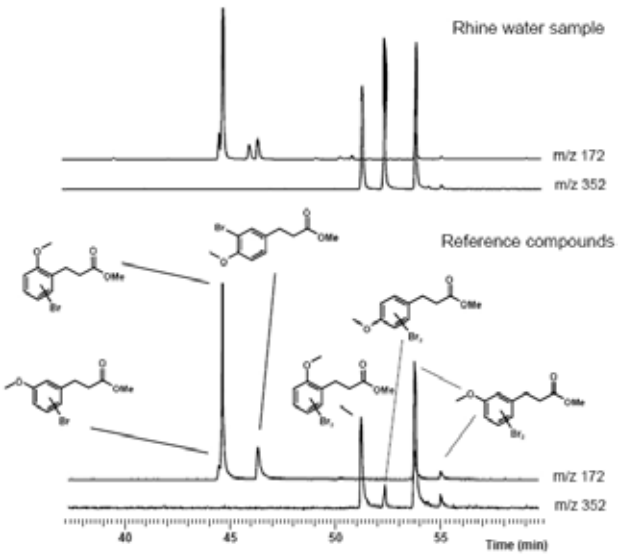

Figure 2: Brominated contmainants identified in Rhine water (according to ref.[8])

Screening analyses on longitudinal section of the rivers Rhine and Lippe (Germany) revealed complex pattern of anthropogenic contaminants comprising a lot of still unnoticed pollutants (e.g. N-butylbenzenesufonamide NNBS, see Fig. 1.) or still unidentified compounds such as brominated aromatics as depicted in Fig. 2.

These investigations demonstrated the need to expand our analytical focus on a broader spectrum of organic contaminants, because such comprehensive investigations can build up an adapted base for advanced monitoring studies.

P o i n t v s N o n - P o i n t S o u r c e s

High attention has been given to non-point and multiple source emissions especially to agricultural contamination (e.g. pesticides) and to sewage effluents derived from treatment plants. The contamination by these sources is characterized by a widespread distribution of environmental stable compounds. However, the emission of point sources like industrial effluents exhibit frequently higher concentration of contaminants and a more complex mixture of substances. Nevertheless, these contaminations are normally restricted to a local importance. Therefore, source point emission are frequently underestimated or neglected, although they can have dramatical impact on river sections.

Investigations performed on industrial emissions contaminating river systems revealed significant contributions of harmful substances. These compounds exhibit frequently molecular structural moieties of high environmental relevance or interest like halogen substitution or aromatic parts see (Fig. 3.). Most of these compounds occurred with elevated concentrations and corresponding loads (examples see Table I) at the outflow from the industrial sites representing a high environmental impact on the connected river system.

Table I
Specific compounds in two different types of industrial effluents and their emission rate

Industry	Compound	Load at outflow [$g\,d^{-1}$]
Petrochemical industry	Triphenylphosphine oxide	>10
Chemical industry	Phenylmethylsulfone	ca 0.5
	2,6-Dithiohexane	ca. 20
	Dichlorobenzenes	ca. 5
	Trichorobenzoic acid	ca. 3

Riverine Particulate Matter

River water phase includes the suspended particulate matter representing an aerobic environment, whereas the sedimentary compartment is mainly more anaerobic. Therefore, particle associated pollutants underly quite different degradation pathways as the result of different microbial communities.

A n t h r o p o g e n i c M a r k e r

In addition to pollutants, characterized by harmful ecotoxicological or toxicological effects, further anthropogenic contaminants were analysed frequently, the so-called ‚anthropogenic markers‘. An impressive review on the evaluation and use of anthropogenic marker compounds has been published[9].

These substances are useful to reflect anthropogenic emissions to natural systems especially in terms of source specificity. Anthropogenic marker compounds are both natural substances derived from human feces or urine and xenobiotics. Apart from information on the emission sources they also provide information on transport pathways and fate of anthropogenic contaminants.

This approach has been applied e.g. to sediment samples from the Lippe river in order to point out the general contamination situation of this riverine system (Kronimus et al. 2005). It was able to differentiate individual industrial emission sources from agricultural and municipal contamination as depicted in Fig. 4.

G e o c h r o n o l o g y

During flood events riverine particulate matter can be deposited on flood plains and riparian wetlands. Accordingly these areas can act as ecological archives in case of undisturbed and periodical sedimentation. The dating of these sediment deposits can be performed by gamma spectrometric analysis of the radio-nuclides ^{210}Pb and ^{137}Cs. Organic geo-

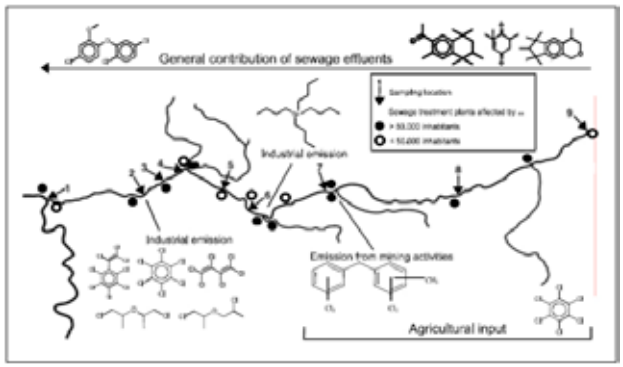

Fig. 4. **Emission situatiuon at the Lippe river as reflected by anthropogenic marker (according to ref.[10])**

chemical investigations on anthropogenic contaminants in these dated layers allow a detailed description of the emission of organic contaminants into the corresponding riverine system[11]. However, this approach is restricted to substances, which are characterized by a sufficient environmental stability and immobility in the particulate phase.

An example was published for the Lipper river system revealing interesting insights into different time periods of contamination and of technical application of additives. Important emission periods are reflected by the geochrnoligical profiles as presenetd in Fig. 5.

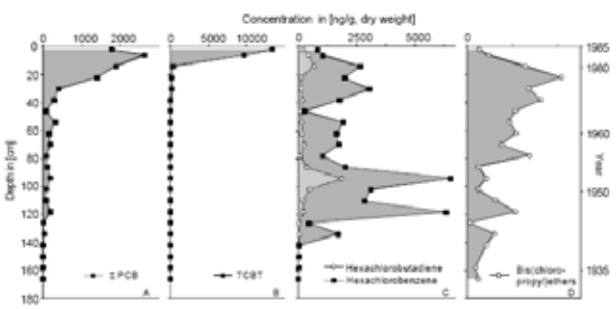

Fig. 5. **Geochronological profiles of selected pollutants in a core from riparian wetlands of the Lippe river (according to ref.[12])**

Carbon Stable Isotopes

Compound specific carbon isotope analyses are increasingly applied in environmental studies. Investigations the aquatic environment focused mainly on polycyclic aromatic hydrocarbons, monoaromatic compounds and halogenated aliphatics.[13–16] Further on, laboratory studies investigated the isotopic fractionation as a result of environmental processes, in particular transport and transformation processes (e.g. vaporization, adsorption, abiotic and biotic degradation) .[17–20] Carbon isotope ratios are not only useful data for evaluating the environmental fate of organic pollutants but also for distinguishing different emission sources discharging the same contaminants.

Interestingly, all applications reported so far avoid the two major limitations of compound-specific isotope analyses: *(i)* the lower sensitivity as compared to traditional GC/MS analyses and *(ii)* the requirement of complete gas chromatographic separation of the contaminants. On the contrary, natural river systems are mostly low or moderately contaminated and, accordingly, concentrations of individual contaminants are also low. Consequently, information on the isotopic characterization of contaminants existing in these water systems are highly restricted.

However, studies dealing with carbon stable isotope values of organic contaminants in Rhine river and Lippe river water samples allowed interesting insights into the environmental fate of selected plasticizers, technical additives and musk fragrances[21,22] (Kronimus and Schwarzbauer, Schwarzbauer.as examplified in Fig. 6.

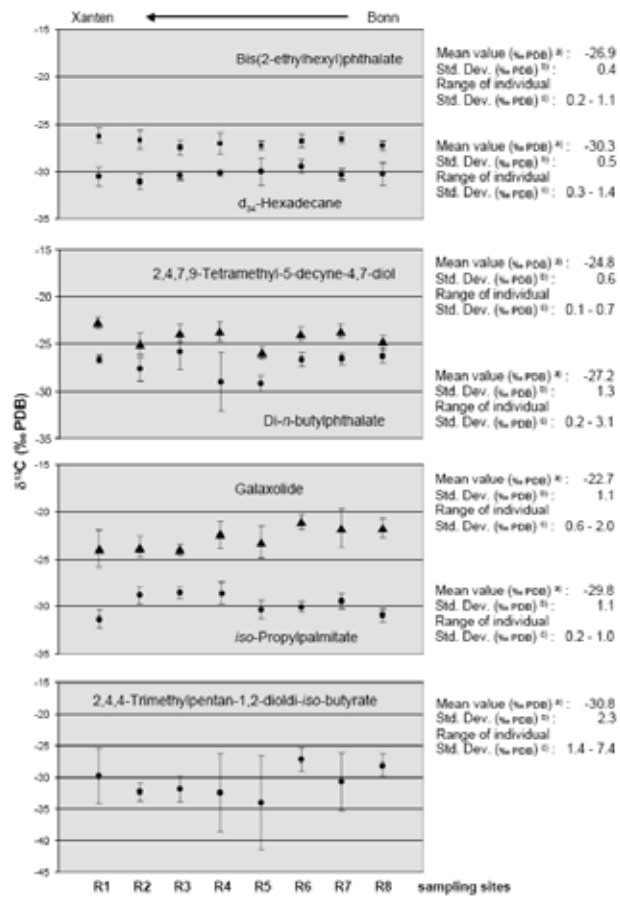

Fig. 6. **Carbon stable isotope values of selected pollutants in Rhine river water (according to ref[21])**

Perspectives

This short overview on recent research activities in the field of riverine organic pollution shall demonstrate the need to broaden the spectra of analytical approaches and strategies in order to meet the challenge to obtain the most detailed and most precious view on the anthropogenic impact on the aquatic environment. Such approaches comprise as described non-target screening, consideration all type of emission source (point/non-point), geochronology, stable isotope analyses as

well as anthropogenic markers. Additionally, also non-mentioned topics like the bound fraction of pollutants immobilised in particulate matter or the contamination by polymers open new insights and expand our knowledge on riverine contaminations and pollutant pathways in rivers.

Noteworthy, major parts of this outline have been recently published more comprehensively as well as in more detail[1].

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L05 CHALLENGES OF THE ANALYSIS OF FOOD AND ENVIRONMENTAL MATRICES BY GC-TOF MS AND GC × GGC-TOF MS

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Introduction

The demands on the analysis of organic contaminants' residues have been increasing recently, taking into account the number of compounds analysed in a single run, number of matrices handled within one method, requirements on detection limits as well as requirements on the analysis speed. Accordingly, considerable progress in both sample preparation techniques and in the instrumentation has been done recently. As for the instrumental part, gas chromatography still remains a widely used separation technique in such residue analysis, thanks to its high separation potential and wide range of applicability. Mass spectrometry is becoming to be used more as primary detection technique in GC, instead of being a confirmatory tool only.

Time-of-flight mass spectrometry offers several important benefits over conventional (scanning) MS detectors: *(i)* With TOF MS full mass spectral information is available across the whole chromatogram, while the achieved detectability is comparable to the one typical for conventional MS detectors. Therefore, unambiguous confirmation of analyte identity is possible according to full mass spectrum. *(ii)* The absence of spectral skewing and the constant ratios of ions across a chromatographic peak allow the application of software data mining algorithms of peak finding and true signal deconvolution. This enables the applications for non-target screening analysis. *(iii)* Being the fastest MS detector, time-of-flight can be used for the applications in fast and ultra-fast GC and comprehensive two-dimensional gas chromatography (GC × GC), as discussed below.

Within the data mining software routines the Peak Find algorithm automatically and effectively locates all peaks in the chromatogram. Co-eluting analytes, peaks buried beneath the background of the TIC, and even small peaks buried beneath large matrix interferences are automatically detected and reported. After the Peak Find algorithm has located all of the analytes, the true mass spectra for each analyte are automatically extracted from the system background, matrix background, and co-eluting components. Even ions that are shared between co-eluted analytes are accurately proportioned to provide a complete mass spectrum. The resulting spectrum is then used for analyte identification by spectral interpretation or by library search using any number of commercially available or operator-prepared databases. Fig. 2. shows the overlap of the tolylfluanide and penconazole peaks with a time difference of 0. s between the peak apexes. Although, it is impossible to distinguish the two peaks in the combined TIC, the peaks can be resolved by plotting the individual ion masses. Apex non-deconvoluted spectra A) contain some ions originating in co-eluted peak (red and green arrows). After applying the deconvolution agorithm these spectra are then missing all these contaminating ions B) and can be then identified by matching to mass spectral libraries C). This ability to separate and identify closely eluting peaks decreases the need for highly resolved peaks and permits faster analysis.

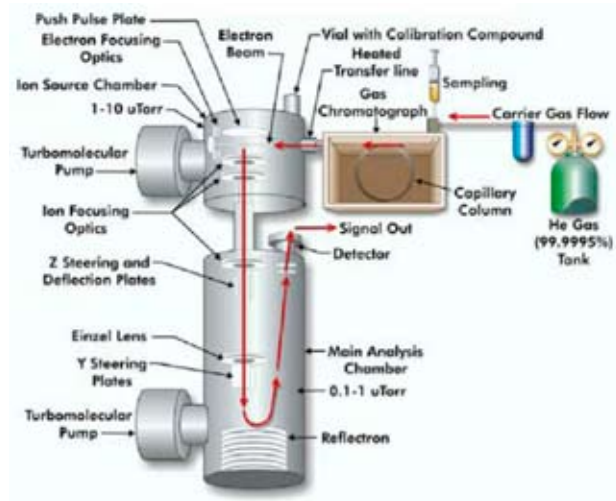

Fig. 1. **TOF MS design (Pegasus HT, LECO, USA)**

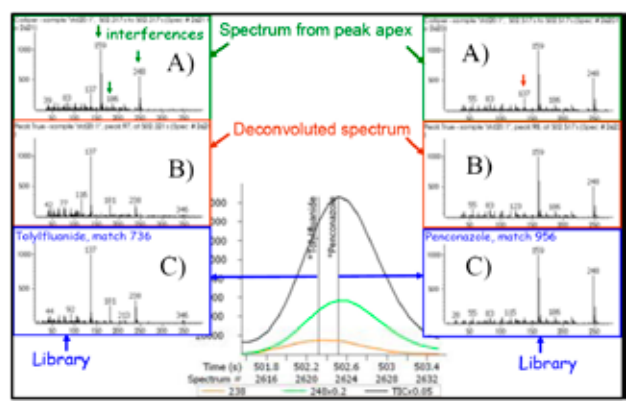

Fig. 2. **True signal deconvolution demonstrated on closely co-eluted peaks of penconazole and tolylfluanide**

Comprehensive two-dimensional gas chromatography (GC × GC) has been a subject of attention in recent years, because of its enormous separation ability. In GC × GC, two columns of different selectivity are connected via a thermal modulator, which cuts small parts of the first column eluate, focuses them to sharp zones and samples them onto the second column, where a very fast "flash" separation (100–150 times faster compared to separation in first column) is performed. Each compound of a sample is therefore subjected to two independent separation mechanisms (see Fig. 3.).

The considerably increased peak capacity of GC × GC over one dimensional GC provides not only improved sepa-

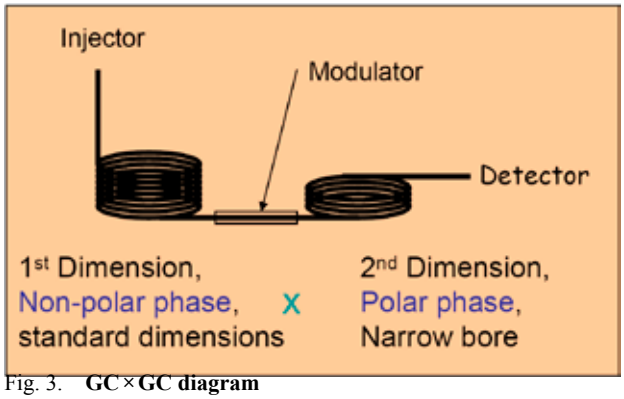

Fig. 3. **GC×GC diagram**

ration of target analytes (see Fig. 4.) but also target analytes from matrix which allow more accurate identification and quantification of analytes.

Results

In the presented contribution applications focusing on GC-TOF MS and GCxGC-TOF MS examination of difficult environmental samples will be shown.

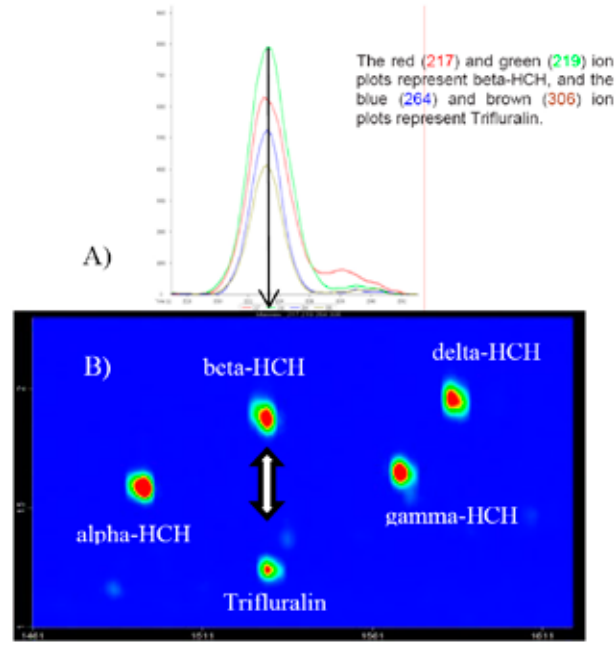

Fig. 4. **Contour plot showing chromatographic separation of beta-HCH and Trifluralin in the second dimension as noted by the white arrow (B), however, they co-elute in the first dimension (A) (ref.[1])**

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L08 TRANSFORMATION OF PETROLEUM POLLUTANT IN THE ENVIRONMENT

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Introduction

Petroleum and its refining products continue to be one of the most abundant environmental pollutants. Consequently, transformation processes of petroleum-type pollutants in soils, recent sediments, alluvial sediments, ground and surface waters were studied by numerous authors: in coastal marine environment[1], in estuarine sediments[2], under arctic marine conditions[3], on the East Mediterranean coast[4] etc.

The fate of an oil type pollutant in the environment can be monitored most accurately by determining its quantity and studying its composition in the polluted samples from the same or close locality, and during different periods of time. On that way a number of experiments were carried out in order to define the intesity and optimal conditions for the most efficient biodegradation (temperature, humidity, nutrients) e.g., [5-11].

On the other side, simulation of the natural conditions in the laboratory or by *ex situ* bioremediation with simultaneous intensification of only specified factors affecting the intensity and rate of transformation made it possible to save time and to come to relevant conclusions. Applications of biodegradation processes foccussed primarily on bioremediation of different sections of the environmental compartments have been described, e.g.[12–14]

In this paper our recent results will be reviewed observed by investigating tranformation processes of petroleum-type pollutant 1) by determining its quantity and studying its composition in the polluted samples from different distance of oil spill; 2) by determining its quantity and studying its composition in the polluted samples during different periods of time from oil spill; 3) by simulation of the natural conditions in the laboratory and 4) by *ex situ* bioremediation with simultaneous intensification of only specified factors affecting the intensity and rate of transformation.

Tranformation Processes of Petroleum Pollutant by Determining its Quantity and its Composition in the Samples from Different Distance of Oil Spill

In order to gain insight into oil transformations which occur during migration and to contribute to the elucidation of migration mechanisms in water-wet environments, the content and composition of heavy fuel oil from an accidental oil spill near a railway station was determined in crushed rock samples from various depths and distances from the oil spill1[5]. The observed differences in the group composition (increase in NSO-compounds content with depth and distance), and in the distribution of n-alkanes and isoprenoids C_{19}, pristane (Pr) and C_{20}, phytane (Phyt) (the shift of the n-alkane-maximum and the shift of Pr/n-C_{17} and Phyt/n-C_{18}-ratios towards higher values with depth and distance; Fig. 1.) lead to the conclusion that heavy fuel oil migration through the crushed rock environemnt occured in the presence of water by the colloidal micelle mecahnisms. Namely, higher n-alkane homologues and pristane and phytane incorporated into the micelles of petroleum NSO-compounds and water and thus they are "transported" preferentially, compared to shorter-chain n-alkanes.

Tranformation Processes of Petroleum Pollutant by Determining its Quantity and its Composition in the Samples During Different Periods of Time from Oil Spill

The process of biodegradation of petroleum-type pollutants in underground waters from Danube alluvial sediments (the locality of Pančevo Oil Refinery) was followed through a period from November 1997 to February 2000 by GC analyses of isolated alkane fractions[16,17] (Fig. 2.).

In the period from November 1997, when first samples were taken, to February 2000, when fifth samples were taken, important changes of the chemical composition were obvious, especialy in well I. Relative contribution of n-alkanes as compared to pristane and phytane in sample Ia indicated changes defined as "initial petroleum biodegradation".

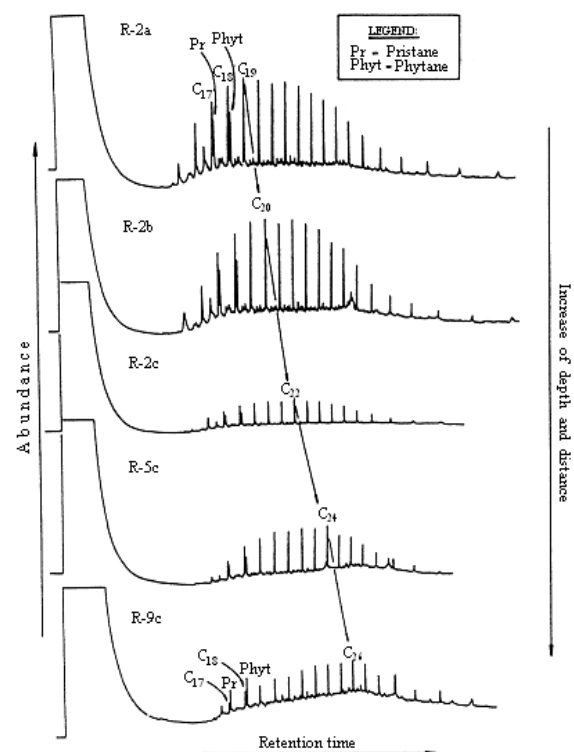

Fig. 1. **Gas chromatograms of saturated hydrocarbon fractions isolated from emptied heavy fuel oil (R-2a → R-9c, increase the depth and distance from oil spill)[15].**

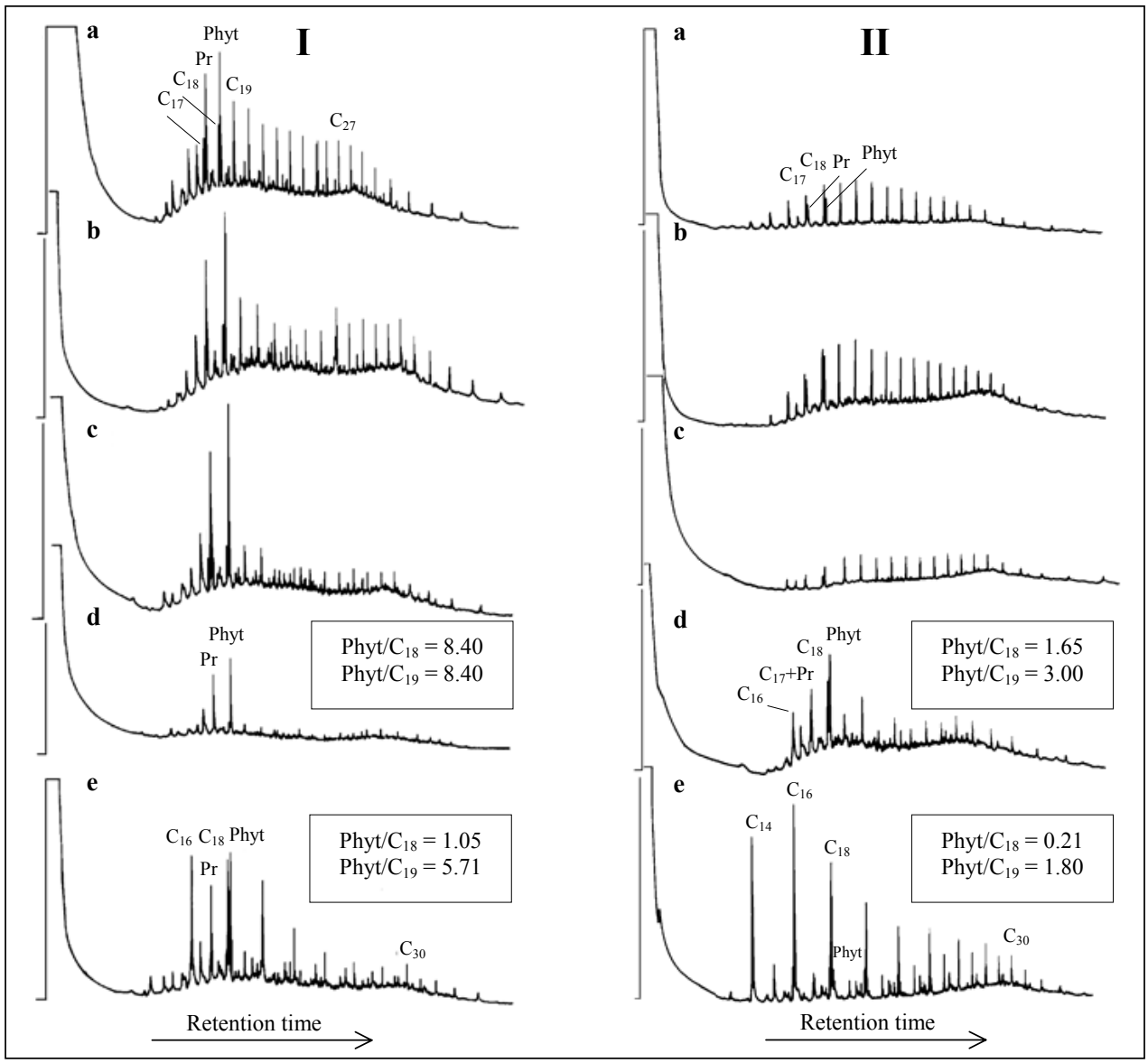

Fig. 2. **Gas chromatograms of alkanes isolated from oil polluted alluvial ground waters (Pančevo Oil Refinery locality, I and II wells). Samples were taken in November 1997 (a), May 1998 (b), in September 1998 (c), September 1999 (d) and in February 2000 (samples e)[16,17].**

The abundance of C_{17} and C_{18} n-alkanes was somewhat smaller than the abundance of pristane (C_{19}) and phytane (C_{20}). Gas chromatogram of sample Ib showed that in the period from November 1997 to May next year the amount of n-alkanes relative to isoprenoids was reduced, a phenomenon typical for biodegradation intensity in geochemical literature defined as "very slight" or "minimal biodegradation"[18–20]. Later, in September 1998 (sample Ic), the amount of n-alkanes was still smaller. Finally, during next one year, n-alkanes were almost completely degraded (sample Id). Pristane and phytane remained nonbiodegraded.

Comparison of Pr/n-C_{17} and Phyt/n-C_{18} ratios observed in samples Ia-c (winter 1997 – autumn 1998) suggested that biodegradation was considerably more intensive during the summer period than during the winter or spring periods. On the other hand, by comparing these degradation intensities with those observed in samples originating from close but deeper localities, it was concluded that biodegradation of the petroleum-type pollutant was more intensive in shallow underground waters[21].In a relative short period of time, from September 1999 to February 2000, the alkane fraction of the petroleum-type pollutant suffered an unexpected change (sample Ie, Fig. 2.). Namely, while pristane and phytane were found in the same amounts characterized by approximately the same ratios, in this fraction of the pollutant new even carbon-number C_{16} to C_{30} n alkanes were observed. It was

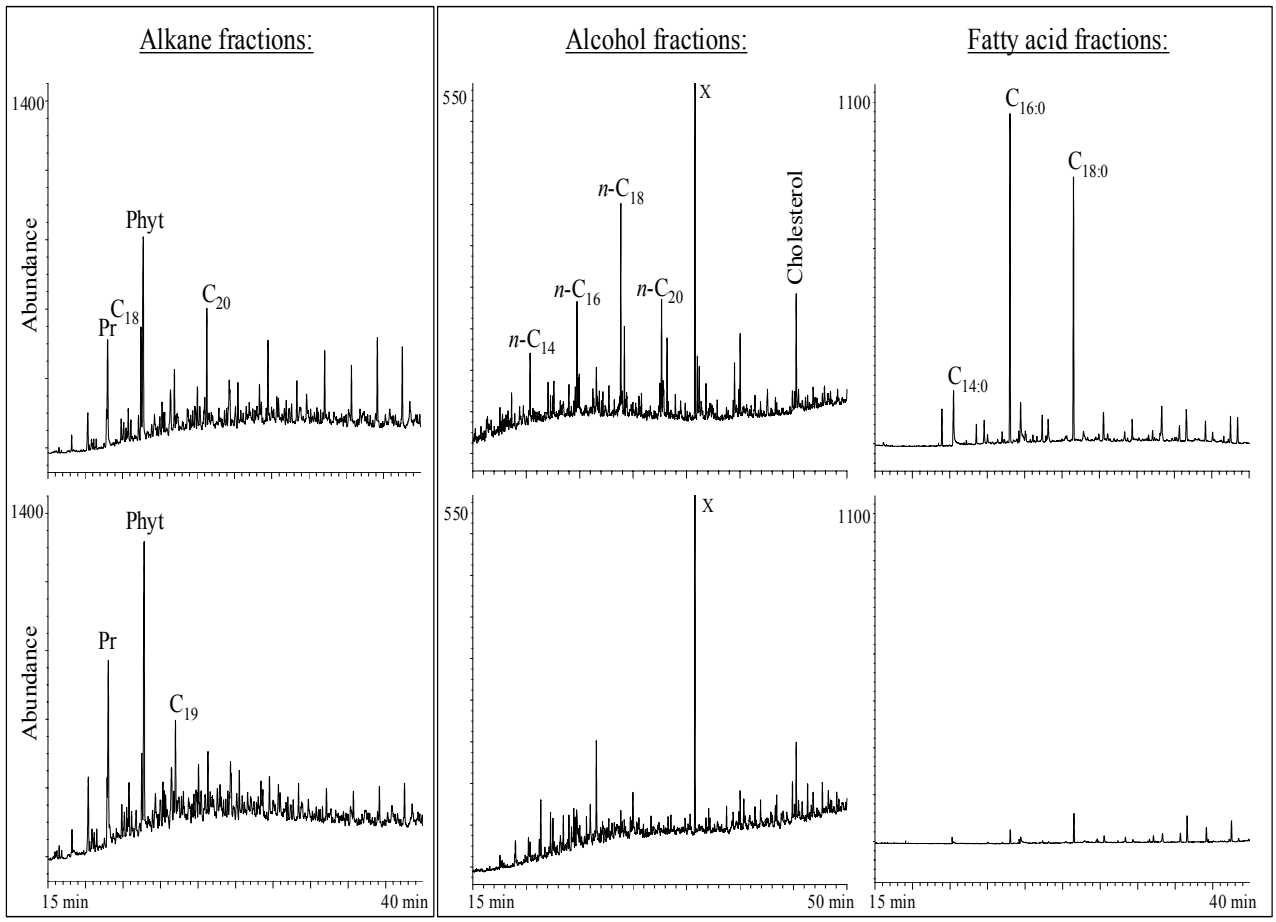

Fig. 3. **Chromatograms of GC-MS analyses of fractions of alkanes, alcohols and fatty acids (their methyl-esters), isolated from extracts I d (below) and Ie (above)[24].**

supposed that these even carbon-number n-alkanes were biosynthesized by some microorganisms. According to literature data, the following organisms are known to synthesize such compounds: Desulfovibrio desulfuricans, Corynebacterium sp., Escherichia coli, Rhizopus stolonifer or Penicillium sp.[22]. These are all micro-organisms which have grown on different organic basal medium (for example sewerage waste[23]). Consequently, the biosynthesis of even carbon-number C_{16}–C_{30} n-alkanes was tried to confirm by detailed analysis of extracts obtained from samples d and e[24] (Fig. 3.).

It was found that sample Ie, containing remarkable amounts of even *n*-alkane homologues, contained, as well, in the alcoholic fraction, a homologous series of even carbon-number alcohols in a C_{14} to C_{20} range and a relatively significant amount of cholesterol. On the other hand, sample Id, which did not contain any significant amounts of neither odd nor even n-alkane homologues, did not contain alcohols or higher fatty acids.

Even carbon-number alcohols and fatty acids observed in sample Ie were taken as a proof of the presence of particular microorganisms, i.e., of unicellular, nonphotosynthetic algae of *Pyrrophyta* type. These types of microorganisms, also known under a popular name of "fire algae", are known to be able to synthesize even *n*-alkane homologues on a suitable base such as petroleum or petroleum-type pollutants[25].

Tranformation Processes of Petroleum Pollutant by Simulation of the Natural Conditions in the Laboratory

The fate of a petroleum-type pollutant in environmental water may be foreseen on the basis of laboratory simulation experiments of microbiological degradation of petroleum using microorganism consortiums similar to those typical for the natural environment, activated on a corresponding nutrient base[26]. As an example, Figure 4 shows a gas chromatogram of the alkane fraction of a paraffinic-type crude oil originating from Serbia oil field (Sir-1C), and gas chromatograms of alkane fractions of the same crude oil isolated after 90 days of simulated biodegradation on an inorganic "Knop" base ($Ca(NO_3)_2 \cdot 4H_2O$, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, KCl, $FeCl_3$, H_2SO_4, H_2O; pH ≈ 8) under daylight (Sir-1) and in absence of light (Sir-2), as well as on a "Bujon" organic base (tryptone, yeast extract, glucose, distilled water, pH ≈ 7) under daylight (Sir-3) and in darkness (Sir-4). The experiments were carried out with microorganisms consortium similar to that one iden-

tified as dominant in the investigated surface sewage water in the channel of the Pančevo Oil Refinery (*Phormidium foveolarum, Achanthes minutissima, Nitzschia communis, Chlorella communis*)[27].

In an experiment on a Knop base, which maximally correspond to natural conditions, by biodegradation under daylight (Sir-1), n-alkanes were almost completely degraded. In darkness, the degradation was less effective (Sir-2). In experiments on a Bujon base, *n*-alkanes were found to be much less degraded (Sir-3 and Sir-4).

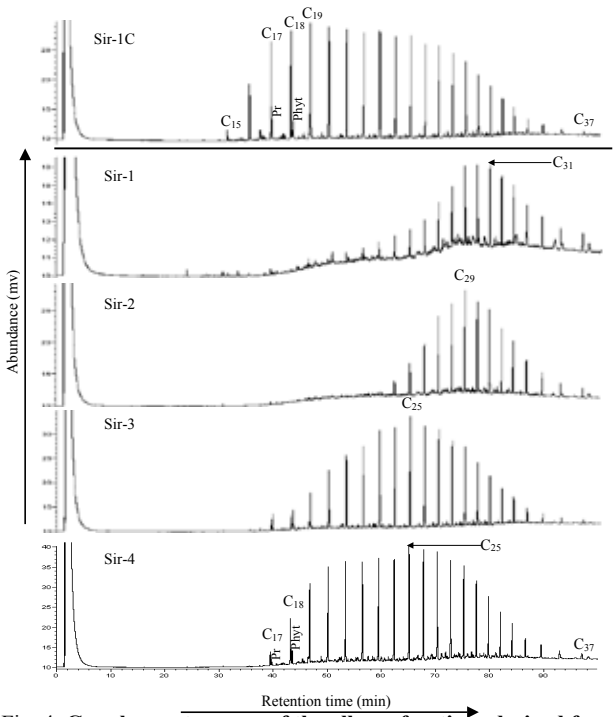

Fig. 4. **Gas chromatograms of the alkane fractions derived from paraffinic type petroleum of Sirakovo after 90 days of simulated biodegradation with *Phormidium foveolarum*, *Achanthes minutissima*, *Nitzschia communis* and *Chlorella communis* with Kp medium in the light (Sir-1), with Kp medium in the dark (Sir-2), with Bh medium in the light (Sir-3), with Bh medium in the dark (Sir-4) together with chromatogram of alkane fraction typical for the control experiments (Sir-1C)[27].**

On the other hand, in experiments carried out in the same way, using the same nutrient bases and identical microorganisms, but with a naphthenic-type crude oil originating also from Serbia, it was possible to follow the degradation of isoprenoid aliphatic compounds27 (Table I). Comparison of Phyt/C30-hopane ratios observed in the investigated samples after 90 days of simulated biodegradation (Ve-1 – Ve-4) with those observed in control tests (Ve-1C – Ve-4C; Table I), showed the degradation of isoprenoids to be also most pronounced on the Knop inorganic nutrient base and under daylight. Under identical experimental conditions, polycyclic alkanes of sterane and triterpane types retained their original distributions.

Table I
Parameters based on gas chromatograms of naphthenic-type crude oil alkane fractions[27]

	Pr/Phyt	Phyt/C30-hopane
Ve-1C	0.21	1.68
Ve-1	ND	ND*
Ve-2C	0.37	3.81
Ve-2	ND	ND*
Ve-3C	0.46	4.00
Ve-3	0.52	1.67
Ve-4C	0.10	0.50
Ve-4	0.30	0.32

ND – parameter was not calculated due to total degradation of pristane and phytane
ND* – parameter was not calculated due to total degradation of phytane

The experiment of *ex situ* soil bioremediation was performed at the locality of the Oil Refinery in Pančevo (alluvial formation of the Danube river, Serbia) polluted with the oil type pollutant[28]. The experiments of biostimulation, bioventilation and reinoculation of autochthonous microbial consortium were carried out during the six-month period (May – November 2006). The changes in the quantity and composition of the pollutant, or the bioremediation effect, were monitored by analysis of the samples of the polluted soil taken in time spans of two weeks. In that way, from the beginning until the end of the experiment, 12 samples were collected and marked with P_1–P_{12} (Pančevo 1 – Pančevo 12). The results obtained showed that more significant changes in the composition of the oil pollutant occurred only during the last phases of the experiment (P_8–P_{12}; Fig. 5.). The activity of microorganisms was reflected in the increase of quantity of the polar oil fractions, first of all fatty acid fractions. In that way the quantity of total eluate increased, and the quantity of the insoluble residue was reduced to minimum, by which the oil pollutant was transferred to a form which can be removed more efficiently and more completely from the soil, as a segment of the environment.

Also, these experiments presented atypical transformations of saturated hydrocarbons of petroleum type pollutants[29]. Namely, organic substance from P_1, P_8 and P_{12} samples were extracted by Soxhlet's method and quantified. Isoprenoid aliphatics, in particular pristane and phytane, and polycyclic aliphatics of sterane and triterpane types in saturated hydrocarbon fractions were analysed by GC-MS (SIM method). Significant amounts of *n*-alkanes have not been detected. The MS-chromatogram revealed only marginal amounts of pristane and phytane in sample P_1. Pristane and phytane occurred in sample P_8, and in even higher quantities in the final sample P_{12} (Fig. 6.). The proceeding bioremediation process was accompanied by the decrease of the relative amounts of pentacyclic terpanes of hopane type, compared to tri- and tetracyclic terpanes[29]. In the initial sample P_1 the distribution

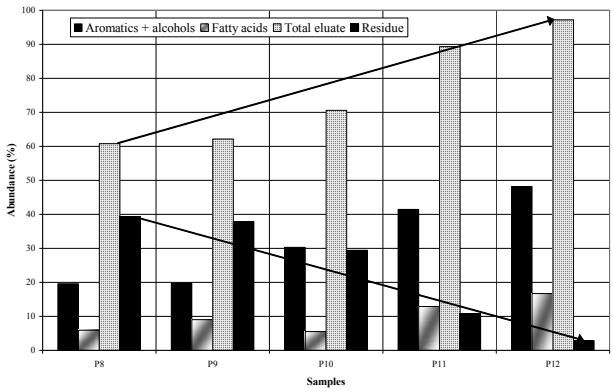

Fig. 5. **Contents of total aromatics and alcohols, fatty acids, total eluate and the column residue for samples P_8–P_{12} (ref.[28]).**

of steranes and hopanes follows a pattern, which is characteristic for crude oils. However, their identification by SIM method was not possible in samples P_8 and P_{12} because of the reduced concentration[29].

The observed changes in the alkane fractions' compositions may be considered as atypical, referring to the fact that during oil biodegradation under natural conditions, decomposition of isoprenoids occurs much easier and faster than decomposition of polycyclic alkanes of tri-, tetra- and pentacyclic terpane, sterane and diasterane types, after the decomposition of *n*-alkanes has been almost completed.

Instead Conclusions

In spite of remarkable advancement of petroleum exploitation, transport and refining technologies, petroleum and its refining products continue to be one of the most abundant environmental pollutants. Consequently, studies on the environmental fate of petroleum-type pollutants remain to be an actual scientific interdisciplinary problem.

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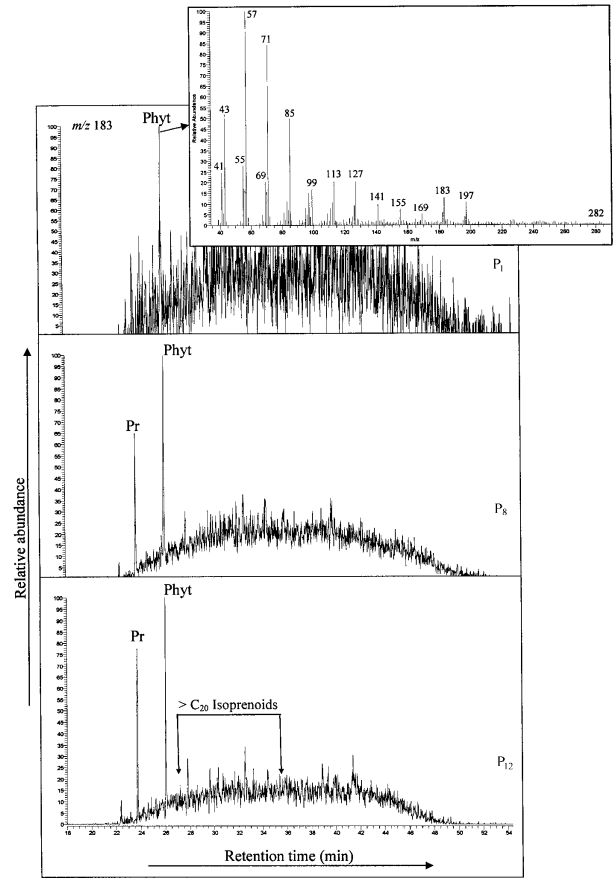

Fig. 6. **Ion chromatogram characteristical for isoprenoids (m/z 183) of samples P_1, P_8 and P_{12} (full mass spectra of phytane peak for P_1 sample is also given)[29].**

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