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P. K. Anokhin Institute of Normal Physiology
Russian Academy of Medical Sciences
Moscow, Mokhovaja 4

USEFUL ADAPTIVE EFFECT
AS A PHYSIOLOGICAL PROCESS IN FUNCTIONAL SYSTEMS OF THE ORGANISM

ABSTRACT: In the present work physiological principles and processes have been considered with respect to the theory of functional systems developed by Academician P. K. Anokhin. The elaboration of these principles, as well as the results of previous experiments, allowed us to propose a new notion of useful adaptive result. The adaptive effect is considered as a physiological process in the central nervous system, which results from reverse afferentation concerning the characteristic of an external object to neurons in the result-accepting apparatus. New notions of the useful effect (regulation of blood pressure, feeding behavior and human social activity) were evidenced by logical and experimental data. We reviewed the neurophysiological processes that accompany goal-directed activity of animals and underlie the formation of useful adaptive result in the system.

KEY WORDS: functional system, adaptive effect, reverse afferentation, result-accepting apparatus, blood pressure, feeding behavior

Recent experiments performed in our laboratory as well as the logical principles of the theory of functional systems allowed us to propose a new notion of useful adaptive result. This notion arose from manuscripts of P. K. Anokhin: "The result (as a system-forming factor) may be considered as a physiological process, which is developed upon the influence of a complex of reverse afferent pulses as the result of action and comparison of the result with a general purpose of this action" [2].

The principle of self-regulation is a general principle of the theory of functional systems (TFS) and classic physiology. The scheme of self-regulation objectivizes any systemic activity of the organism (from regulation of homeostatic function to human social activity) [10]. Figure 1 represents 3 levels of self-regulation in humans: blood pressure (BP), feeding behavior and social activity. These schemes include 2 units. According to TFS, one of these units is the adaptive result: a) blood volume that determines the pressure in the aorta; b) food from the external environment; and c) a videocassette containing a video film.
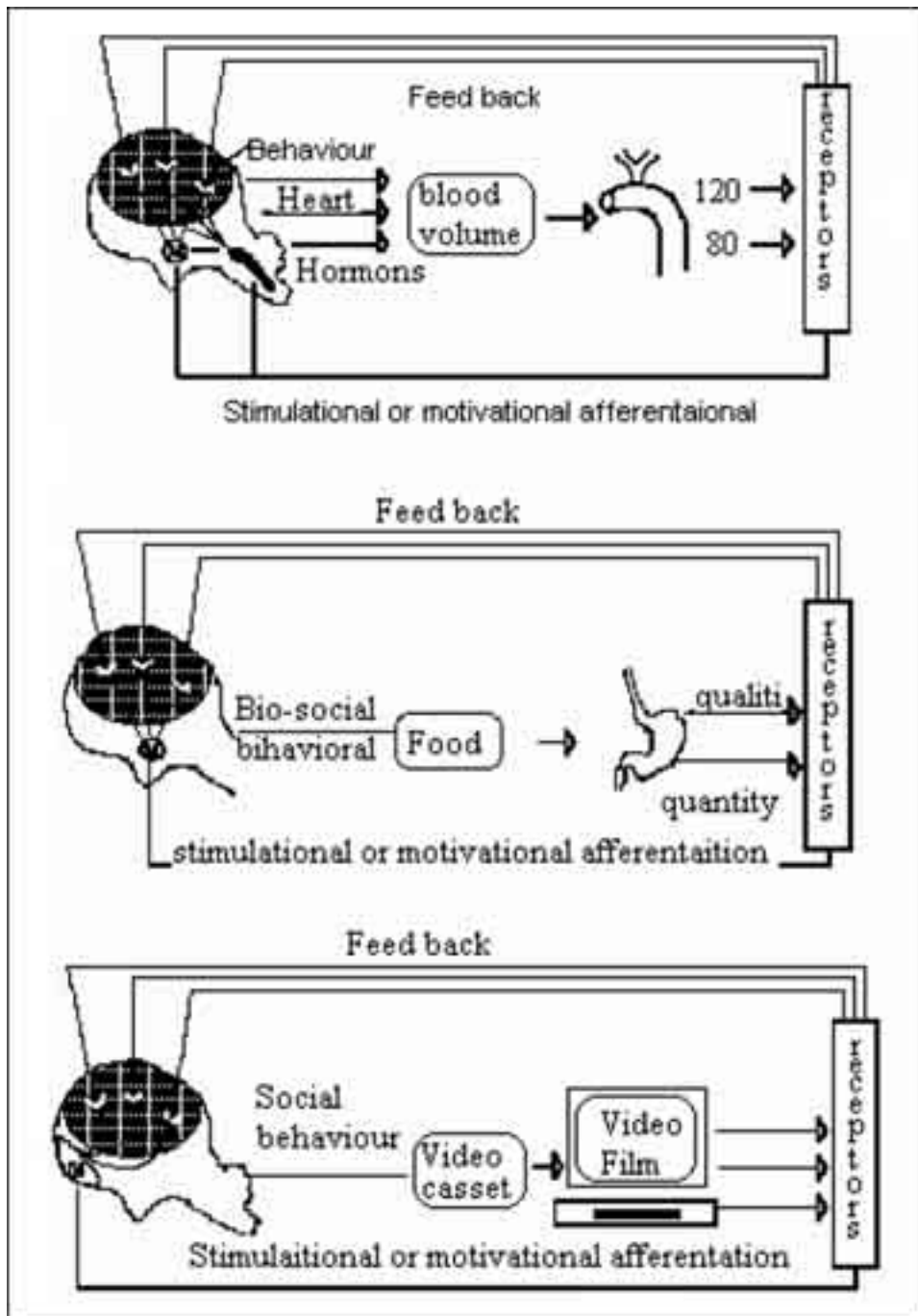
Fig. 1 — A scheme of self-regulating functional systems of the homeostatic, bio-social and social levels of the human organism

Unit 1: external objects (relative to functional systems), results of action of this system. Unit 2: interaction between reverse afferentation concerning the characteristic of an external object and apparatus in the central nervous system (acceptor of the action result, AAR), which predicts and evaluates the performance of a specified action program and parameters of a required external object satisfying the basic motivation.

The TFS postulates that this interaction may lead to an agreement or a discrepancy in AAR. It results in the appearance of a positive or negative emotion, respectively. The following two questions arise. First: is the result (as a system-forming factor) localized within or outside the system? Second: once the emotions are formed during evaluation of the end adaptive result, does the interaction in AAR serve as a result?

To answer these questions, it is necessary to remember the TFS. P. K. Anokhin believed that "a researcher investigating behavioral activity of animals and humans primarily focuses on this activity, but not on the results of activity". This study was performed within the framework of a reflex theory. P. K. Anokhin proposed to evaluate not only activity, but also the results of behavioral activity (*i.e.*, following events in the external environment). Modern notions of the functional system and system-forming factor are based on these
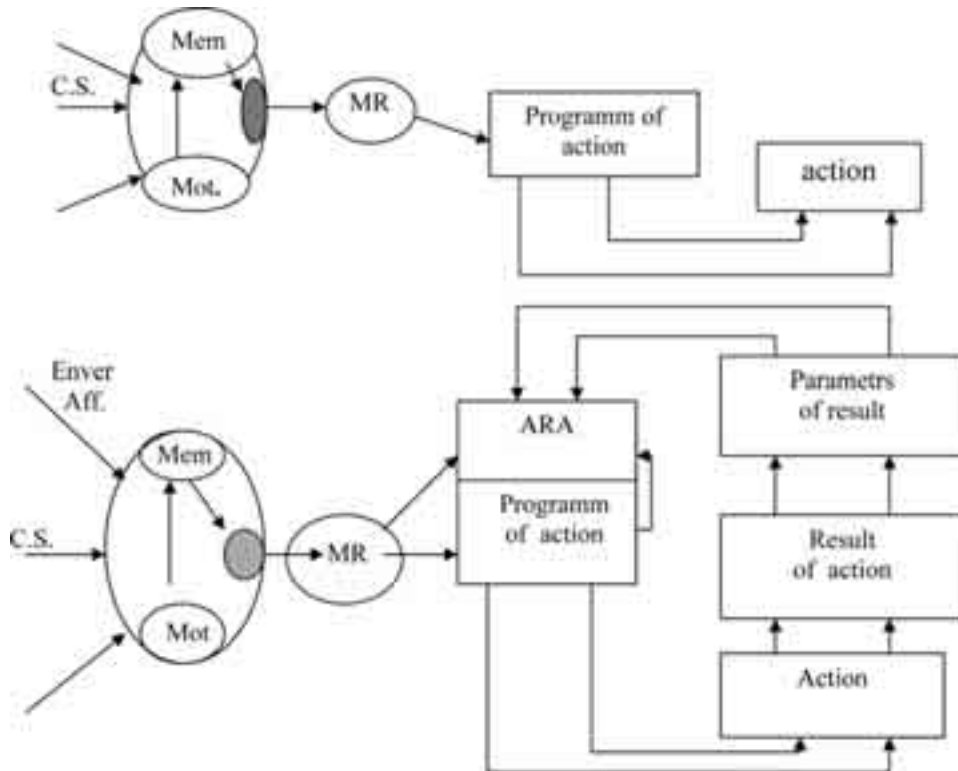
Fig. 2 — A logical scheme of goal-direction behavior

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logical principles and results of studying the mechanisms for compensation of impaired functions [2].

In the present work we attempted to elaborate the logical principles P. K. Anokhin applied in the study of behavioral activity. Attention was paid to processes in the external environment and the resulting events in the system. The TFS postulates that the organism with dominant motivation and specific goal (acceptor of the action result, AAR) aims to interact with the results of activity and evaluate them by means of AAR. This procedure allows us to evaluate whether the results of activity satisfy the basic motivation. The evaluation is a physiological process which determines the organism's emotional state (Figure 2).

An important determination of the result is that "the end adaptive effect serves as a central component in the functional system of any level of organization" [2]. This definition of the adaptive result ("effect" in the system) is additional logical evidence that during the evaluation of BP, food, movie, etc., the useful adaptive effect should be considered as a physiological process in the system.

A possible place of the emotional mechanism for evaluation of the result in the architectonics of the functional system remains unclear. P. K. Anokhin wrote that this mechanism evolved in nature as a set of neurophysiological, neurochemical, and molecular processes. They form an emotional state to evaluate the utility of "effects" in the system. We shall attempt to introduce this mechanism into the scheme of the functional system.

Another theoretical background to characterize the useful adaptive result is the principle of advanced reflection of reality. It is realized via the mechanisms of motivation and genetic and acquired memory in the central nervous system. These mechanisms form a special apparatus to evaluate activity of functional systems. Moreover, they form a physiological basis to adjust activity and achieve the end adaptive effect.
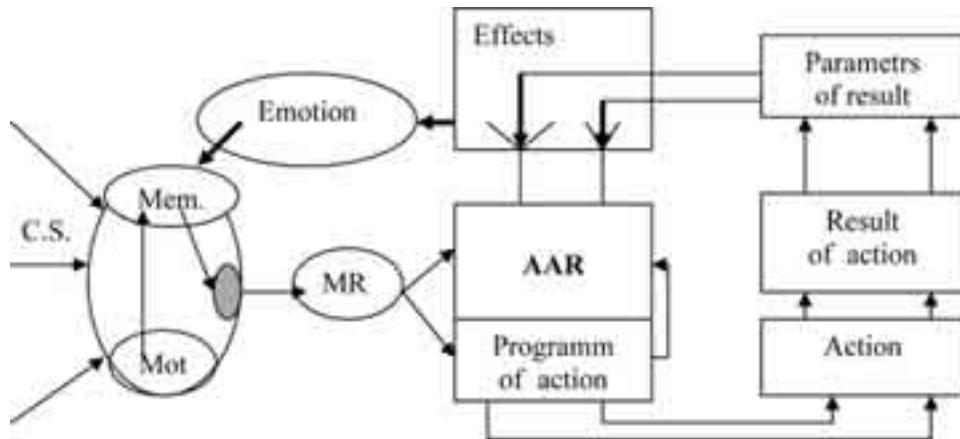
Fig. 3 — A new scheme of functional systems of goal-directed behavior. The useful adaptive result as a physiological effect or the process of relationship between parameters of result and acceptor of the action result.

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We believe that this physiological basis includes an actual interaction between the nervous apparatus in CNS and reverse afferentation concerning the performance of a specified action program as well as changes in the external environment ("results" of action).

Thus, the "effect" results from an interaction between central neurons with reverse afferentation concerning the characteristic of an external object or event (Figure 3). Our assumptions are confirmed by the results of experimental study (see below).

RESULTS

Experiments were performed on freely moving rabbits on models of feeding behavior and defense behavior. We recorded the locomotive activity of the animals, extracellular spike activity of nerve cells in various brain structures, respiration and electrocardiogram (ECG).

There are many approaches to interpreting the results of studies (G. Jasper; recent experiments to evaluate neuronal activity during animal's behavior) [1, 3, 4, 14]. We believe that these interpretations are related to 2 reasons: (1) "historical" transfer of the notion that neuronal activity reflects an organism's reaction to various factors (first microelectrode studies were performed on narcotized animals) [12]; (2) consideration of neuronal activity as activation, inhibition or no change in the discharge frequency per time unit.

However, our studies revealed no difference in the average discharge frequency for 100 lateral hypothalamic neurons (center of hunger) in fasting animals and 100 neurons in satiated animals.

Another approach suggests studying a change in interval characteristics of the pulse activity of neurons. We revealed a similar distribution of modes in various brain structures of animals (reticular formation; lateral and ventromedial hypothalamus; insular, visual and sensorimotor cortex; hippocampus; globus pallidus; thalamus; etc.) under a specific motivational state.

Experiments with rabbits after 48 h starvation showed that 34 neurons in the lateral hypothalamus exhibit activity under conditions of food presentation before satiation. Study of the interval pattern of neurons in fasting animals (comparison of histograms) revealed the bi- and trimodal distribution. The duration of dominant intervals corresponded to 5—10, 150 and more than 100 msec. Discharge activity of neurons appeared as groups or trains of pulses [6].

On the one hand, the interval pattern of neurons reflected dominant motivation of hunger in animals. On the other hand, it illustrated "anticipation" of the future food result [5]. A. I. Shumilina believed that a discrepancy in AAR without the actual result would lead to the state of strain or negative emotion. The negative emotion reflects a "negative" result in the system, which is manifested in a specific discharge activity of neurons.

With respect to the architectonics of the functional system for the behavioral act, group or train activity of neurons reflects the existence of dominant motivation, operation of AAR and negative emotional state.

Further experiments showed that feeding activity of animals is accompanied by a significant change in the interval pattern of neuronal activity. Group

activity was transited to regular and monotonous activity. The interval histogram was characterized by the unimodal distribution and a variety of dominant intervals for various cells.

These data indicate that the neuronal activity (regular activity) serves as an objective criterion for agreement in AAR and positive emotional state when the animal achieves a specific goal. A principal question arises concerning the adaptive result (effect) from the viewpoint of a researcher (food intake) or organism (operating system).

Transition of neurons to regular spike activity follows reverse afferentation to central neurons (relative to food characteristics) and may be used as an objective criterion of the positive effect in the system.

Further experiments were performed on freely moving rabbits. We recorded extracellular activity of neurons in various brain structures, including the reticular formation; lateral and ventromedial hypothalamus; insular, visual, and sensorimotor cortex; hippocampus; globus pallidus; thalamus; etc. [8].

A study was performed on a model of the conditioned feeding response. We revealed general principles of the change in the interval pattern of brain neurons.

When the animals engaged in the feeding activity, the train activity of neurons in various brain regions was shifted to regular activity. These changes in various brain structures were observed in different periods of time: start of rabbits' movement to a feedbox (realization of the action program, sensorimotor cortex); appearance of food in an automated feedbox (visual cortex); food intake (insular cortex); and food transition to the stomach (hypothalamic structures).

This study indicates that a specific temporal structure of the pattern for discharge neuronal activity becomes regular during the interaction of reverse afferentation from receptors responsible for realization of the action program and reacting to various characteristics of the external object (food). Our experiments and previously published data show that these interactions not only occur in receptors of the gastrointestinal tract, but also involve the process of cell metabolism [11].

We analyzed the effectiveness of animal behavioral activity. It was necessary to compare the following terms: (1) "reinforcement" during acquisition of a conditioned response (I. P. Pavlov); and (2) "result" as a system-forming factor (P. K. Anokhin).

The model of defense behavior involves unconditioned reinforcement with electrocutaneous stimulation (ECS). It is difficult to explain this model from the viewpoint of TFS. Avoidance of ECS is the adaptive result under these conditions. However, ECS is followed by the formation of this functional system.

We performed a special series with acquisition and decrement of a conditioned response in rabbits. Acoustic stimulation for 6 seconds served as a conditioned stimulus. Reinforcement suggested electrocutaneous stimulation of the hind limb with 14 mA for 1.5 sec. Experiments were conducted on awake rabbits fixed to a machine by the limbs.

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Study of 82 neurons in the dorsal hippocampus revealed specific changes in the interval characteristics of individual cells (98% cells) produced by 40 combination exposures to acoustic stimulation and ECS. Besides this, specific features were found upon a decrement in the response (40 acoustic stimulations) [9].

We studied the baseline activity of neurons in immobilized animals. The duration of dominant intervals corresponded to 1—50, 150, and more than 100 msec. It reflects the formation of defense motivation [7]. Regular activity appeared 1000—1200 msec after the first 3 exposures to ECS. These findings are of particular interest since similar changes in the interval characteristics of neurons occur in fasting animals during food reinforcement. On the one hand, ECS probably serves as a reinforcing stimulus. On the other hand, ECS is followed by afferentation to neurons producing the phenomenon of "result" (criterion of the interval pattern). However, avoidance of ECS is usually considered as a positive result in TFS. It may be suggested that neurophysiological mechanisms decreasing the sensitivity to ECS (increase in the threshold, activation of the opioidergic system, etc.) are triggered in the organism under conditions of unavoidable pain stimulation.

Particular attention was paid to neuronal activity after termination of ECS. Regular activity is followed by the appearance of trains with the bimodal distribution of intervals (1—50 and 150 msec). A. I. Shumilina showed that the animal anticipates pain stimulation even after termination of ECS. No stimulation produces discrepancy and, therefore, increase in the strength of defense motivation [14].

Combination exposure to conditioned stimulation and ECS is followed by several stages of changes in the interval pattern of hippocampal neurons. In stage I (5—7 combinations) train activity was observed during conditioned stimulation. It reflected the initial step of conditioned response acquisition and anticipation of future of ECS ("advance", P. K. Anokhin). Long-lasting regular activity was revealed after ECS. In stage II (10—14 combinations) regular activity developed in response to conditioned stimulation and ECS. In stage III (14—20 combinations) regular activity was revealed before and during conditioned stimulation. The inhibition of spike activity was found after ECS. Under conditions of follow-up combinations, spike activity was blocked or increased before ECS. The observed changes are similar to those accompanying automated forms of behavioral activity in animals [12, 13].

Study of interval characteristics showed that the learning process in animals is accompanied by a change in the pattern of neuronal activity. It reflects two principles of TFS: advanced reflection of reality and minimization of functions (discharge activity of neurons).

Regular activity of neurons is of particular interest. Interval characteristics of neuronal activity were similar to those observed after the start of individual ECS and remained unchanged during combination exposure to acoustic stimulation and ECS. We hypothesized that the organism achieves the positive effect under these conditions, which is related to the activity of internal neurophysiological processes of adaptation to electrocutaneous stimulation. The neurons receiving afferentation from pain stimulation, pain conditioned stimulus

and ECS and operating as a part in the acceptor of action results tend to exhibit the regular activity. It reflects the achievement of a positive adaptive effect.

These data indicate that the adaptive effect of systemic behavioral activity may be associated with the interaction of afferentation from external objects (exposure) with neurons in the acceptor of actions results.

CONCLUSION

In the present work we tried to advance the notions of "result" in TFS of P. K. Anokhin. One of these aspects suggests that it is necessary to develop the theory of P. K. Anokhin. Which changes are observed by a researcher when studying the activity of animals and humans? What is the result of this activity? The study should be focused not only on the results of the activity but also on the processes in the system that occur after the achievement of these "results". This approach allows us to consider previous notions of the action result and evaluate the useful adaptive effect (interaction of afferentation with neurons in the acceptor of action results).

These data give an interpretation of the complex social activity of humans and they solve the problem of hierarchy in functional systems (hierarchy of results).

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КОРИСТАН АДАПТАЦИОНИ ЕФЕКАТ У ФУНКЦИОНАЛНИМ СИСТЕМИМА ОРГАНИЗМА КАО ФИЗИОЛОШКИ ПРОЦЕС

Борис Васиљевич Журављев

Научно-истраживачки институт нормалне физиологије имена П. К. Анохина, Руска Академија медицинских наука, Москва, Мокховаја 4.

Резиме

У раду су изложени физиолошки принципи и процеси са становишта теорије функционалних система П. К. Анохина.

На основу развоја тих представа, а исто тако добијених експерименталних резултата, предлаже се нов појам о корисном адаптационом ефекту. Адаптациони ефекат се разматра као физиолошки процес који се одвија у централном нервном систему на основу пристизања аферентација о параметрима спољашњег објекта неуронима укљученим у структуре апарата акцептора резултата дејства.

Изнети су логички и експериментални докази нове представе о корисном ефекту на нивоу регулације крвног притиска, исхрамбеног понашања и социјалног облика активности човека. Размотрени су неурофизиолошки процеси при усмереној активности животиња, који се налазе у основи формирања корисног адаптационог ефекта у систему.

D u b r a v k a   S.   M i l a n o v[1]
L j i l j a n a   Đ.   S u v a j d ž i ć[2]

[1] Scientific Veterinary Institute "Novi Sad"
   Rumenački put 6, 21000 Novi Sad, Serbia
[2] Faculty of Medicine, Department of Pharmacy
   Hajduk Veljkova 3, 21000 Novi Sad, Serbia

CHARACTERISTICS AND IMPORTANCE OF THE GENUS *PROTOTHECA* IN HUMAN AND VETERINARY MEDICINE

ABSTRACT: *Prototheca* spp. are strange algae, assigned to the genus *Prototheca*, family *Chlorelaceae.* They are ubiquitous in nature, living predominantly in aqueous locales containing decomposing plant material. *Prototheca* spp. were isolated from skin scarificates, sputum and feces of humans in absence of infection, as well as in a variety of domestic and some wild animals. *Prototheca* spp. are unicellular organisms, oval or spheric in shape. They differ from bacteria and fungi in size, shape and reproductive characteristics. Of the five known species of the genus, only *P. wickerhamii* and *P. zopfii* are considered pathogenic, and they are the only known plant causative agents of human and animal infections. Over the past 25 years medical references reported more than 100 cases of human prototothecoses, mostly induced by *P. wickerhamii* and rarely by *P. zopfii*. A half of the reports on human prototothecoses relates to localized cutaneous infections and oleocranon bursitis. The rarest and most severe form of the infection is disseminated or systemic protothecosis, described in patients with durable course of primary disease or immune disfunction. In veterinary medicine, *Prototheca zopfii* and rarely also *P. wickerhamii* are reported as causative agents of cutaneous protothecosis in dogs and cats, systemic protothecosis in dogs and mastitis in dairy cows. Protothecal infections are diagnosed by histopathology examination or, more exactly, by isolation of the agent, although the organism cannot be distinguished from the yeasts by its cultural characteristics. Final diagnosis is made by the carbon-hydrate assimilation test. Protothecal infections are easily missed in routine practice. Pharmacological protocol for therapy of this rare infection has not been developed yet either in human or in veterinary medicine. Several antifungal agents are aplied for treatment; however, the effects are variable. Where possible, surgical excision is treatment of choice. Prognosis is promising in patients with localized infection, and healing is often achieved. Prognosis is less predictable, mostly bad, in patients with other diseases and in immunocompromized patients. Sensitivity of *Prototheca* spp. *in vitro* does not necessarily correlate with its efficacy *in vivo*.

KEY WORDS: *Prototheca* spp., Clinical signifikance, Diagnosis

In 1880, Z o p f and K ü h n first isolated unknown organisms from the slime flux of a linden tree. The isolates were morphologically and physiolo-

gically characterized in 1894 by K r ü g e r, who identified them as fungi on account of their cultural similarity to yeasts (K r ü g e r, 1894). The organisms were identified as algae in 1913 due to their ability to sporulate similarly as *Chlorella* species, and reclassified three years later by West (W e s t, 1916) to the genus *Prototheca*, family *Chlorelaceae*. *Prototheca* is an achlorophyllous mutant of the green algae with nonfunctional chloroplast. Achlorophyllous mutants of *Chlorellae* have been successfully produced in the laboratory; although the pathway of their genesis in natural environment still remains unclear (H u s s and S o g i n, 1990). Until now, five species have been generally recognized within the genus: *P. wickerhamii, P. zopfii, P. moriformis, P. ulmea* and *P. stagnora*.

DISTRIBUTION

Prototheca spp. is ubiquitous in nature and widely distributed all over the world. The organism had first been isolated from the slime flux of trees and over the following decades from a wide variety of sources including sewage, soil, plants and many fresh and salt water sources, even from water-supply systems (A n d e r s o n and W a l k e r, 1998; P o r e et al., 1983, W a l s h et al., 1998). The organism is particularly predominant in aqueous locales containing decomposing plant material. *Prototheca* spp. were isolated from skin scarificates, sputum and feces of humans in absence of infection (S o n c k and K o c h, 1971), as well as in a variety of domestic and some wild animals. Occurrence of the organism in the feces of cattle, pig, horses and sheep is associated with the use of contaminated feed.

Life cycle and morphological traits

Prototheca spp. are monocellular organisms, oval or spherical in shape, 7—16 μm in diameter (A n d e r s o n and W a l k e r, 1998; D i P e r s i o, 2001). They differ from bacteria and yeasts in size, shape and reproductive characteristics. Contrary to yeasts and bacteria they do not have glucosamine in their cell wall and do not contain muraminic acid, respectively (D i P e r s i o, 2001). The cell wall of *Prototheca* consists of outer (thinner) and inner (thicker) envelope, while all *Chlorella* species (except for *Ch. prototecoides*) are characterized by a three-layer cell wall (S u d m a n, 1974). The three species commonly isolated from the natural environment are considered non-pathogenic, i. e., *P. stagnora, P. ulmea* and *P. moriformis*. These species are able to produce capsule (similarly to *Cryptococcus neoformans*); however, this feature was not observed in any of clinical isolates of *P. wickerhamii* and *P. zopfii* (D i P e r s i o, 2001). The reproduction is asexual, by internal septation and endospore formation. The sporangia contain 2—16 or more daughter-cells (sporangiospores), which, following the characteristic cell-wall breakage, further develop the endosporulating cells (P o r e, 1998).

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Prototheca spp. as causative agent of infection

Of the five known species of the genus, only *P. wickerhamii* and *P. zopfii* are considered pathogenic, yet their pathogenic potential is low. Until now, they are the only known plant causative agent of human and animal infections (R o e s l e r et al., 2003).

Human protothecoses

The first case of human protothecosis was described by D a v i e s et al. in 1964, manifested as localized skin lesions in a farmer in Sierra Leone, while the first systemic protothecosis was described by C o x et al. in 1974. Over the past 25 years, medical references reported on more than 100 cases of human protothecoses, mostly induced by *P. wickerhamii* and rarely by *P. zopfii* (K r c m e r y, 2000; M o n o p o l i et al., 1995; W i r t h, 1999). Though un-frequent, prothecal infections are reported worldwide: in Europe, Asia, Africa and Central America. In our country, the first isolation of *Prototheca* spp. from clinical material was reported on April 5, 2005 by the Serbian Micro-biology Association, Section of Vojvodina (S u v a j d ž i ć, 2005). Infections occur in all age categories, but extremely rarely in the pediatric population.

Described as environment-borne agent, *Prototheca* spp. enter the body via the traumatic skin lesion and mucous membranes, on subsequent exposure to contaminated water. Human-human transmission route is excluded. The incu-bation period has not been precisely defined yet; however, anamnestic data on post-traumatic infection suggest a 2-week incubation period.

Some 50% of all reports on human prototheces relate to localized cuta-neous infections, slowly-developing single or multiple lesions that do not heal spontaneously. They are described as diffuse erythema, papulae, vesico-pustu-les, ulcerations, nodous, eczematous or herpetiform changes located mostly on extremities. Pain and swelling of soft tissues may occur, as well as various quantities of serous-sanguineous liquid. Regional lymph nodes are rarely affec-ted. Failure of prompt diagnosis of cutanous protothecosis may result in the development of chronic destructive lesions even in immunocompetent indivi-duals. The second most frequent form of protothecosis is olecranon bursitis, which develops as a consequence of traumatic implantation of the agent. It is manifested as induration of the bursa, associated by swelling and moderate erythema (G a l a n et al., 1997). The rarest and most severe form of the infec-tion is disseminated or systemic protothecosis, described in patients with dura-ble course of primary disease or immune dysfunction (*diabetes mellitus*, mali-gnancy and chemotherapy, renal transplantation, systemic *lupus erythematosus*, corticosteroid therapy and HIV infection) (K u n o v a et al., 1996).

Animal prototheceses

In veterinary medicine, *Prototheca zopfii* and rarely also *P. wickerhamii* are reported as causative agents of cutaneous protothecosis in dogs and cats,

systemic protothecosis in dogs and mastitis in dairy cows (G o n z a l e s, 1996, M o n o p o l i et al., 1995).

Dogs and cats

Cutaneous protothecosis induced by *P. wickerhamii* is the only disease manifestation described in cats (D i l l b e r g e r et al., 1988). In both cats and dogs, the cutaneous form results from injury infection and is manifested by the occurrence of ulcerative lesions, scabs and pyogranulomatous dermatitis in limbs, trunk and mucous surfaces. Hyperkeratosis may develop too, and frequent complications are due to secondary bacterial infections. Pathohistological examination of skin bioptates reveals abundant protothecal organisms within the cytoplasm of phagocytic cells (G i n e l et al., 1997).

In systemic protothecosis in dogs it is most likely that algae enter the body by ingestion, passing the intestinal mucosa (infection portal) and disseminating to the entire body via the hematogenic and lymphogenic routes (H o l - l i n g s w o r t, 2000). Systemic prorothecosis in dogs is mostly induced by *P. zopfii*, rarely by *P. wickehamii*, and disease symptoms depend on the organs and organ-systems affected, as well as on the severity of the lesions. Unremarkable and unspecific symptoms mostly result in delayed diagnosis, enabling the agent to spread over the entire body. Even in systemic infections only cutaneous manifestations, symptoms of gastro-intestinal disorders and eye infection were clinically manifested (H o l l i n g s w o r t, 2000; G i n e l et al., 1997).

Numerous reports have indicated gastrointestinal disorders as most frequent clinical signs of disseminated protothecosis. M i g a k i et al. (1982) described hemorrhagic colitis in dogs induced by *P. zopfii.* The colon is the most severely affected site, but lesions are visible over the entire intestine. Clinical manifestation of the disease includes vomiting, tenesmus and intermittent diarrhea (blood and slime in the feces). Colonoscopy reveals diffuse hyperemia, hemorrhages and ulcerations, as well as multiple granuloma of the mucosa, which, in the further course of the disease, results in intestinal stricture and obstipation.

The infection can spread to the central nervous system, cardio-vascular system and urinary tract, liver, skeletal muscles, lymph nodes, thyroid gland, pancreas, peritoneum and diaphragm (H o l l i n g s w o r t, 2000). Infection of the eye is common manifestation of systemic protothecosis in dogs. It results in severe damages and blindness that are due to development of glaucoma and retinal ablation. *Prototheca* are identified by microscopy examination of *humor vitreus* (S c h u l t z e et al., 1998). Manifest signs of disease in CNS (cervical pain, depression, ataxia, pareses) are rare. The affected organs exhibit typical reaction to protothecal infection, i.e., formation of granulomes 0.5—2 mm in diameter with the cell infiltrate composed of cell plasma, macrophages and neutrophiles.

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Prototheca was first linked to mastitis by L e r c h in 1952. Mastitis in dairy cows is mostly induced by *P. zopfii*, sometimes by *P. wickerhamii* (G o n - z a l e s, 1996; J a n o s i et al., 1998). *P. zopfii* mastitis is a disease of highly productive cows in the machine-milking systems. Although it mostly occurs sporadically, endemic events are also reported (J a n o s i et al., 2000). *Prototheca spp*. as well as species of *Staphylococcus, Streptococcus, Arcanobacterium pyogenes* and *Mycoplasma* may be of great significance for the herd (B o b o š and V i d i ć, 2003), especially if infection is not timely diagnosed. Similarly to some bacterial species such as *Arcanobacterium pyogenes, Pasteurella multocida* or *Corynebacterium ulcerans*, *Prototheca* is rarely identified as the mastitis agent (S u v a j d ž i ć et al., 2001b; S u v a j d ž i ć et al., 2003; S u v a j d ž i ć et al., 2001). The wide distribution of these algae in the environment and their occurrence and isolation at the farms showing no sign of prothothecal mastitis indicate the crucial role of predisposing factors, such as poor milking hygiene or prolonged antimicrobial therapy of mastitis of other etiologies (A n d e r s o n and W a l k e r, 1998; S c h l e n d s t e d t et al., 1997; T e n h a g e n et al., 1999). Outbreaks of seasonal prothothecal mastitis have been reported during warm and humid periods of the year that promote the propagation of these organisms in their natural habitat (C o s t a et al., 1996).

Infection commences at the papillae of mammary glands in all lactation phases, including dry period (www.Uvex.edu/miklquality/prototheca). In the first weeks of lactation an increased susceptibility to infection was observed, which is due to the pronounced energetic misbalance (C o s t a et al., 1997). The infective dose is not yet defined. In experimental conditions, infusion of 40—480 CFU *P. zopfii* results in mastitis in 100% cases. Five days after the experimental infection the alga can be isolated from milk in a yield 20 000—50 000 CFU/ml (M c D o n a l d et al., 1984a). Anticipated infective doses for natural infection are high in comparison with other mastitis agents. This quantity is provided through permanent contact of teeth ends with the primary infection sources such as soil, plants, water sources and feces. A common infection source is improper sanitation before the mastitis treatment, since the organism may be introduced via the infusion material, similar as *Nocardia* species. Furthermore, cow-to-cow infection during milking is also possible (A n - d e r s o n and W a l k e r, 1998; D i o n, 1979). *Prototheca* spp. have been recovered from the rubber parts of milking equipment and they showed height resistance to routine disinfection with chlorine solutions (A n d e r s o n and W a l k e r, 1998; C o s t a et al., 1997). Despite the observed mixed infections with *Staphylococcus aureus* and *Streptococcus* spp., synergy of *P. zopfii* and other bacteria pathogenic for bovine udder was not confirmed (S c h l e n - s t e d et al., 1997).

Prothothecal infections result in chronic manifest subclinical and clinical mastitis without signs of systemic infection (fever, depression) (T e n h a g e n et al., 1999). Compared with mastitis forms of other etiologies, the inflammatory process is milder during the acute phase. Besides the moderate course

of the disease and pain, decrease in milk production is observed, associated with somewhat altered, watery secretion containing clots. The usual antimicrobial therapy brings no improvement. Somatic cell count is mostly higher than 1 million/ml; however, individual values may range between 6 and 9 millions/ml (J a n o s i et al., 1998), sometimes even exceeding 20 millions/ml (M a l i n o w s k i et al., 2002). In that respect, even a limited infection may affect the milk quality within herd. Pursuant to our Regulations on Hygienic Safety of Milk, the threshold value for somatic cell count in hygienically safe milk is 500,000/ml, while regulations of EU countries do not allow values higher then 300,000/ml (B o b o š et al., 1997). If prototheccal infection, especially subclinical one, is present in a herd, the somatic cell count in bulk milk samples may be the indicator of the suspect agent.

Since there is neither effective therapy nor spontaneous cure, protothecal infections of the mammary gland become chronic and persist through several lactating periods. Algae are limited to the mammary gland and regional lymph nodes exhibiting granulomatous inflammation. Histological lesions are characterized by massive aggregation of macrophages, plasma cells and lymphocytes (B o b o š and V i d i ć, 2003). Sporangia and sporangiospores are inside the macrophage and neutrophiles in the alveolar lumen and interstitium, suggesting that intracellular proliferation is responsible for inability to overcome the infection (J a n o s i et al., 1998). A pathohistological examination of udder tissue in chronic disease course has revealed the progressive interstitial mastitis with consequent alveolar atrophy (J a n o s i et al., 2001). Such changes of the mammary gland result in a progressive drop in milk production (C o s t a et al., 1997; M c D o n a l d et al., 1984b; T e n h a g e n et al., 1999).

Attempts to locate sites containing *Prototheccae* in dairy-cow housings have not proved to be successful, as examination of highly-contaminated samples from the environment does not support such measure (www.Uvex.edu/mikl-quality/prototheca). Cows should be kept out of humid areas, particularly these containing manure and decomposing plant material in order to prevent the papillae from exposure to the infection sources (C o s t a et al., 1996). Proper draining system will lower the number of microorganisms in the environment. It is of paramount importance to prevent contact of the teeth ends with feces and moist manure 25—30 minutes after milking because of the relaxed papilla sphincter (J a n o s i et al., 1998). If *Prototheca* is isolated from milk samples, it is recommended to examine all cows in the herd (H o d g e s et al., 1985). Similar to the *Mycoplasma bovis* and *Staphylococcus aureus*, *Prototheccae* are intermittently shed in the milk (G o n z a l e s, 1996). As they may be transferred from cow to cow during milking, it is necessary to separate the infected animals and milk them last until final exclusion from the herd (A n d e r s o n and W a l k e r, 1998). Sporadic occurrence of mastitis justifies the culling of infected animals as a measure for prevention of further spread of the infection.

A failure to promptly diagnose the infection and to apply measures for prevention of its spreading inevitably results in the culling of infected animals and decreased milk production and quality, which may cause significant economic losses at dairy farms. The program for mastitis control must include also the *Prototheca* algae (S c h l e n s t e d et al., 1997). Pursuant to legislative regulations in our country, only the exclusion of *Staphylococcus aureus* and

Streptococcus agalactiae is obligatory. As identification of protothecal infections is not a part of routine microbiological examination in veterinary medicine, reports on their occurrence are extremely sporadical (M i l a n o v, 2004; M i l a n o v, 2005). A more complex diagnostic approach including a longer incubation period and use of restrictive media should become a regular practice in diagnosing clinical and subclinical mastitis (S u v a j d ž i ć et al., 2001a).

Milk and dairy products contaminated with *P. zopfii* are potential sources of human infection. An examination of the susceptibility of 40 strains of *P. zopfii* isolated from cow milk to different heat-treatment regimens (72—75°C/ 15 sec; 72—75°C/20 sec and 62—65°C/30 minutes) revealed resistance in at least one of the tests in 34 of the examined strains (M e l v i l l e et al., 1999).

Diagnosis

Protothecoses are diagnosed by histopathological examination or, more exactly, by isolation of disease agent.

Histopathological examination

Prototheca spp. are haematoxilinophyllic, hence, staining of tissue section by haematoxilin-eosin is not the most appropriate method for their identification. Visualization of the organism is achieved by *Gomori-methenamine silver* or *periodic acid-schift* (PAS) staining techniques when "morulae" are visible, mostly bigger than the ones observed *in vitro* (10—30 μm). Empty sporangia resemble fungi. The sporangia of *Prototheca* spp. are smaller and contain fewer spores in comparison with *Coccidioides immitis* and *Rhinosporidium seeberi*. The organism differs from *Blastomyces dermatitidis* and *Cryptococcus neoformans* by the size, internal structure and lack of budding (D i P e r s i o, 2001). For identification of *Prothecae* in tissue specimens, the immunofluorescency technique is applied (S u d m a n and K a p l a n, 1973).

Isolation

Prototheca spp. can be recovered (*intra vitam*) from samples of skin scarificates (cutaneous protothecosis), joint punctuate (bursitis olecranona), feces (hemorrhagic diarrhea in dogs), urine (urinary infections in dogs) and cow milk (protothecal mastitis). Material is inoculated on blood agar and Sabouraud dextrose agar using standard laboratory techniques. Plates are incubated for 48—72 h at 25—37°C (M i l a n o v et al., 2003q).

Cultural and biochemical features

Prototheca spp. grows easily on most standard laboratory nutritive media such as blood agar and Sabouraud-dextrose agar. For isolation from contami-

nated samples, application of selective media containing inhibitors of normal microbial flora is recommended. The growth of *Prototheca* spp. is inhibited by cycloheximide, yet not by hloramphenicol. A special *Prototheca* medium contains folate for inhibition of bacterial growth and 5-florocytosine for suppression of yeast growth. After incubation for 24—72 h at 25—37°C, visible *P. wickerhamii* colonies are formed which are smooth, white and yeast-like (D i P e r s i o, 2001). Colonies of *P. zopfii* on Sabouraud-dextrose agar are clearly formed in primary culture after 48—72 hours of incubation. They are mostly large, irregularly margined, with granular surface and a compact central protrusion (J a n o s i et al., 1998; M i l a n o v et al., 2004). Colonies grown on blood agar are mostly very small and pale gray (M i l a n o v et al., 2004). If the isolation medium does not contain growth inhibitors, *Prototheca* colonies may be overgrown (covered) by bacteria after prolonged incubation (D i P e r - s i o, 2001). Since it is slow-growing, the organism may easily be missed in routine practice if incubation is terminated after 24 h (T e n h a g e n et al., 1999). The colonies are of creamy consistency, readily suspended in saline solution when making microscopy slides. Microscopy is indispensable to distinguish *Prototheca* spp. from yeasts, since differentiation is not possible on the basis of their cultural features.

Characteristic microscopic appearance of *Prototheca* spp. is best observed by examining native preparations using phase-contrast microscopy, when formations described as "morula" or "mulberry" is visible. *Prototheca* spp. is easily methyl-blue and Gramm-stained, but heat-fixation may induce morphological impairment. In Gram-stained preparations, positively stained spores and Gram-negative empty sporangia are visible (34). Sporangia of *P. wickerhamii* are 7—13 μm, of *P. zopfii* 14—16 μm in diameter. Final diagnosis is made by the carbon-hydrate assimilation test (API 20C Bio Merieux; VITEK® Yeast Biochemical Card; RapID Yeast Plus System-Remel). All *Prothecae* use glucose as the carbon source. Trechalose assimilation is the key parameter for differentiation between the two pathogenic strains. According to phenotype and genetic criteria, including growth- and biochemical characteristics and serotyping, various isolates of *P. zopfii* are classified into three biotypes. Biotype II, which ferments glucose and glycerol but not galactose, is mostly isolated in bovine mastitis and human enteropathies (R o e s l e r et al., 2003).

Therapy and prognosis

Pharmacological protocol for therapy of this rare infection has not been developed yet either in human or in veterinary medicine. Several antifungal agents are applied for treatment; however, the observed effects are variable. In human medicine, Amphotericin B, a product of *Streptomyces nodosus* (S u - v a j d ž i ć, 2004), is used for therapy of disseminated protothecosis. This is a funghistatic or fungicide that inhibits ergosterole synthesis in the cell membrane of *Prototheca* spp. The drug has proven to be nephrotoxic, and confirmed oversusceptibility is contraindication to its use. Administration of immidazoles, such as itracosanole, ketoconasole, fluconasole and clorimazole, may show so-

me improvement; however, their efficacy is not yet confirmed due to variable clinical response. Where possible, surgical excision is the treatment of choice. Prognosis is promising in patients with localized infection, and healing is often achieved. Prognosis is less predictable, mostly bad, in patients with other diseases and in immunocompromised patients.

Therapy of systemic protothecosis in dogs may include amphotericin B, tetracyclin, ketoconasole, itraconasole, fluconasole and chorimazole; however, outcomes of such therapies are not yet established. Cutaneous protothecosis caused by *P. wickerhamii* requires both medicamentous treatment and surgical excision.

In vitro isolates of *P. zopfii* from milk exhibited sensitivity to amphothericin B, nystatin, polymyxin B, gentamicin and neomycin (M c D o n a l d et al., 1984b; M a l i n o w s k i et al., 2002). Effective therapy of protothecal mastitis has not yet been developed and the only measure to control the spread of infection in the herd is exclusion of infected animals.

Sensitivity of *Prototheca* spp. *in vitro* does not necessarily correlate with its efficacy *in vivo*.

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ОПШТЕ КАРАКТЕРИСТИКЕ И ЗНАЧАЈ АЛГИ ГЕНУСА *PROTOTHECA*
У ХУМАНОЈ И ВЕТЕРИНАРСКОЈ МЕДИЦИНИ

Дубравка С. Миланов[1], Љиљана Ђ. Сувајџић[2]
[1] Ветеринарски институт "Нови Сад", Руменачки пут 6,
21000 Нови Сад, Србија
[2] Медицински факултет, Одсек за фармацију, Хајдук Вељкова 3,
21000 Нови Сад, Србија

Резиме

Prototheca spp. су релативно непознате алге, сврстане у генус *Prototheca*, фамилије *Chlorelaceae*. Убиквитарне су природи и њихова распрострањеност је углавном везана за влажна подручја која садрже биљну материју у распадању, али су изоловане и из кожних скарификата, спутума и фецеса људи, као и из

низа домаћих и неких врста дивљих животиња. *Prototheca* spp. су једноћелијски организми овалног или сферичног облика који се од бактерија и гљива разликују по величини, облику и начину репродукције. Од пет познатих врста овог рода, само се *P. wickerhamii* и *P. zopfii* сматрају патогенима и, досада, оне су једини познати биљни узрочни агенс инфекција људи и животиња. У последњих 25 година у медицинској литератури описано је више од 100 случајева хуманих прототекоза, углавном изазваних алгама *P. wickerhamii*, а ређе *P. zopfii*. Око половина свих извештаја хуманих прототекоза односи се на локализоване кожне инфекције и бурситис олекранона. Најређа и најтежа форма инфекције је дисеминирана или системска прототекоза, описана код пацијената са другим основним обољењем или имунолошком дисфункцијом. У ветеринарској медицини *Prototheca zopfii* а ређе и *P. wickerhamii* наводе се као узрочници кутане прототекозе паса и мачака, системске прототекозе паса и маститиса млечних крава. Прототекалне инфекције се могу дијагностиковати патохистолошким прегледом или егзактније, изолацијом узрочника, мада се од квасница не могу разликовати само на основу културелних особина. Дефинитивна дијагноза поставља се тестом угљено-хидратне асимилације. Проблем код ових инфекција је што се на њих ретко посумња и нису предмет рутинске лабораторијске праксе. Ни у хуманој ни у ветеринарској медицини нема дефинисаног фармаколошког протокола у терапији. Неколико антифунгалних агенаса користи се у терапији са различитим ефектом. Тамо где је могуће, с обзиром на локализацију, третман избора је хируршка ексцизија. Код пацијената са локализованом инфекцијом прогноза је добра и обично се постиже излечење. Прогноза код пацијената са другим обољењима и имуносупресијом мање је предвидива и може бити лоша. Осетљивост *Prototheca* spp. на антифунгалне агенсе *in vitro* не мора бити у корелацији са *in vivo* ефикасношћу.

Ljiljana Đ. Suvajdžić[1]
Ekaterina A. Mrđa[2]
Ljubomir D. Džambas[3]
Mirjana A. Bogavac[4]

[1] Faculty of Medicine, Department of Pharmacy
  Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[2] Medical Center "Medlab", Institution for Laboratory medicine
  Ilije Ognjenovića 7, 21000 Novi Sad, Serbia
[3] Faculty of Medicine, Department of Stomatology
  Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[4] Faculty of Medicine, Clinical Center — Department of
  Gynaecology and Obstetrics, Hajduk Veljkova 3
  21000 Novi Sad, Serbia

ISOLATION OF *PASTEURELLA MULTOCIDA* SUBSPEC. *MULTOCIDA* FROM CHRONIC PERIAPICAL LESION

ABSTRACT: This study presents five isolates of *Pasteurella multocida* subsp. *multocida*, isolated from chronic periapical inflammatory lesion. We described the methods of sampling and cultivation as well as diagnostic criteria. *Pasteurella multocida* was diagnosed on the basis of characteristic cultural and tinctorial properties and the facts that all strains produced indole and induced ornithine decarboxilation, glucose, saccharose and manitole fermentation. Isolates produced neither urease, nor fermented lactose and maltose. Further classification to subspecies *multocida* was based on the fact that all investigated isolates fermented trechalose, xylose and sorbitol, the traits which are diagnostically significant for the species. Patients deny any contact with farm animals or pets, which indicates a possible aerosol transport and animal-human as well as human-human infection. We consider that this organism should be paid more attention by dentist, oral surgeons and microbiologists.

KEY WORDS: *Pasteurella multocida*, Periapical Lesion, Izolation

INTRODUCTION

Pasteurella multocida is a commonly isolated animal pathogen (B a - l o w s et al., 1991); however, it is a rarely isolated in humans and its etiology is still unclear. There are reports on isolation from goat respiratory organs, wounds and skin burns (H o , B u s h, 2000). Recently, this rare agent has been implicated in severe systemic illnesses in humans, such as endocarditis (F u k u m o t o et al., 2002), lung abscess (H a z o u a r d et al., 2000), menin-

gitis (W a d e et al., 1999) and septicemia after animal bite (S h i m i z u et al., 1995). Diseases in humans are commonly attributed to the contact with farm animals (I s e n b e r g and D'A m a t o, 1990; S h i m i z u et al., 1995) or pets (A r a s h i m a et al., 1992), since the *Pasteurella* species is recognized as "normal flora" in the oral cavity of cats, dogs and other domestic and wild animals. Human infection can also occur after non-bite or other direct animal exposure; usually by aerosol droplet-route by kissing the pet (A r a s h i m a et al., 1992).

All five patients in whom this microorganism was isolated denied any contact with animals, including pets. In the available literature from our country (P o p o v i ć et al., 1974; S o k o l o v i ć, 1975) as well as from international sources (G r o s s m a n, 1959; S p a t a f o r e et al., 1990) we did not find a single references on isolation of *Pasteurella* species as a causative agent or accidental finding in chronic periapical inflammatory lesion. In that respect, we are of the opinion that our isolation of *Pasteurella multocida* subspecies *multocida* from two radicular cysts and three granulomes positioned around the tooth root is the result worth to be presented.

MATERIAL AND METHODS

Sampling and transportation

Specimens were obtained aseptically, in the operation room. The mucosa of the oral cavity was disinfected with 0.5% asepsole and 76% alcohol. After lifting of mucoperiostal lobe, steel trephine was used to remove buccal cortex of the jawbone and to make an opening wide enough to approach the outer wall of the granuloma or a cyst. A sterile syringe was used for aspiration of the lesion-content, which was then transferred to a thyoglycolate broth and submitted to the laboratory. Specimens were examined immediately.

MICROBIOLOGICAL METHODS

Aliquots of thyoglycolate broth were cultured on agar plates containing 10% sheep blood, one nutritive and one McConkey agar, and Sabouraud medium. Both blood and a nutritive agar were cultivated/inoculated simultaneously with and without Staphylococcus aureus growth line. One of each blood plates were incubated under microaerophillic conditions, while the remaining plates were incubated under aerobic conditions at 37°C, by the method described by Suvajdžić (K a p e t a n o v, 2000). The remaining thyoglycolate broth was cultivated at 37°C and subcultured after 48 hours on blood agar plates. Plates (one of each sample) were cultivated under aerobic, microaerophillic and anaerobic conditions. All primarily inoculated plates were examined after 18 hours of incubation. Colonies suspected to be *Pasteurella* were Gram-stained and subcultured to McConkey agar, blood agar and nutritive agar. Biochemical series included the following: Kligler, liquid indole, urease by Christensen, ornit-

hin, glucose, lactose, saccharose, maltose and mannitol by the method recommended by Quinn (Q u i n n, 1998).

Further differentiation to subspecies was done on the basis of the ability to ferment trechalose, xylose, arabinose, sorbitole and dulcitol. Diagnostic test of susceptibility to penicillin disc was also performed.

RESULTS

After incubation period of 18 hours, visible colonies were observed on two blood agar plates, cultivated under aerobic and microaerophillic conditions. The colonies appeared bigger in conditions of increased CO_2 tension. Isolates did not show any dependence on staphylococcal growth.

Colonial grown on the nutritive agar did not require addition of blood factors for growth.

Colonies were delicate, dew-drop like, grayish, butter-like in consistence, shiny and convex. They were easily removable and dispersible. Neither a complete nor an incomplete erythrocyte lysis was observed. On a nutritive agar, colonies of the same traits grew slightly slower and were smaller than the colonies on the blood agar. No growth of primary cultures or subcultures occurred on McConkey and Sabouraud media.

Subcultures were performed from the thyoglycolate medium after 48 hours of incubation. Growth was observed in aerobic and anaerobic conditions, but the best growth rate was obtained under microaerophillic conditions. Colonies exhibited the same characteristics as the primary isolates. Preparations were made both from primary isolates and subcultures and subjected to Gram--staining technique. In Gram stained preparations, after 18 hours, gracile, short, Gram-negative rods and coccobacils were observed, sizing around 1 micron and showing strong bipolar staining.

All isolates revealed positive reaction for cytochromoxidase and catalase production.

BIOCHEMICAL CHARACTERISTICS

Two biochemical series were made of each specimen — one from the primarily cultivated plate and one from the subculture after incubation in thyoglycolate broth. There were no deviations among the series. All strains produced indole, decarboxilated ornithin, fermented glucose, saccharose and manitol. The investigated isolates showed negative results for urease production and for lactose and maltose fermentation.

On the basis of the presented parameters, the strains were defined as *Pasteurella multocida*. Further classification to three possible subspecies determined the organism as *Pasteurella multocida* subspecies *multocida*, due to the fact that all investigated isolates fermented trechalose, xylose and sorbitol, which are traits diagnostically significant for the species.

DISCUSSION AND CONCLUSION

Our positive findings in oral cavity, upper and lower parts of respiratory organs (H o, B u s h, 2000) indicate the possibility of isolation of this organism in humans, which can cause a severe disease especially in compromised hosts and under conditions of decreased general or specific immunity (in our patients — the preceding dental problems).

With respect to the cultural, tinctorial and biochemical characteristics (S u - v a j d ž i ć, 2000), our isolates corresponded with the available literature (H o l - m e s et al., 1995; Q u i n n, 1998). Since *Pasteurella* was isolated from five patients with a pronounced pathological process, presenting the only bacterial isolate, we believe it was etiologically significant. We are of the opinion that this microorganism deserves particular attention of dentists (sampling and transportation of specimens) and bacteriologists (since its colonial appearance and routine biochemical examination of isolates may misdirect them towards "nonfermenting microorganisms" which present normal bacterial flora or oral cavity or towards weakly fermenting representatives of the genus *Enterobacteriacea*).

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ИЗОЛАЦИЈА *PASTEURELLA MULTOCIDA* ИЗ ХРОНИЧНИХ
ПЕРИАПИКАЛНИХ ИНФЛАМАТОРНИХ ЛЕЗИЈА*

Љиљана Ђ. Сувајџић[1], Екатерина А. Мрђа[2], Љубомир Д. Џамбас[3],
Мирјана А. Богавац[4]
[1] Медицински факултет, Одсек за фармацију,
Хајдук Вељкова 3, 21000 Нови Сад, Србија
[2] Здравствена установа „Медлаб", Завод за лабораторијску дијагностику,
Илије Огњеновића 7, 21000 Нови Сад, Србија
[3] Медицински факултет, Клиника за стоматологију,
Хајдук Вељкова 3, 21000 Нови Сад, Србија
[4] Медицински факултет, Клинички центар — Одсек за гинекологију
и акушерство, Хајдук Вељкова 3, 21000 Нови Сад, Србија

Резиме

У раду је приказано пет изолата *Pasteurella multocida* subsp. *multocida* изоло-ваних из хроничних периапикалних инфламаторних лезија. Описан је начин узимања материјала, бактериолошке обраде узорка и дијагностички критеријум. На основу карактеристичних културалних и тинкторијалних особина, као и чи-њенице да су сви сојеви продуковали индол, декарбоксилисали орнитин, фер-ментисали глукозу, сахарозу и манитол, а ни један испитивани изолат није про-дуковао уреазу, нити ферментисао лактозу и малтозу, постављена је дијагноза *Pasteurella multocida*. Subspecies *multocida* одређена је чињеницом да су сви ис-

питани изолати ферментисали трехалозу, ксилозу и сорбитол. Ни један од пацијената није био у контакту са фармским животињама нити кућним љубимцима, што указује на могућност аеросолног транспорта како са животиње на човека тако и са човека на човека. Мишљења смо да овом микроорганизму треба посветити више пажње од стране стоматолога, оралних хирурга и микробиолога.

L j i l j a n a   Đ.   S u v a j d ž i ć [1]
E k a t e r i n a   A.   M r đ a [2]
M i r j a n a   A.   B o g a v a c [3]
L j u b i š a   D.   D ž a m b a s [4]

[1] Dr Ljiljana Suvajdžić, Faculty of Medicine
   Department of Pharmacy, Hajduk Veljkova 3
   21000 Novi Sad, Serbia
[2] Medical Center "Medlab", Institution for Laboratory medicine
   Ilije Ognjenovića 7, 21000 Novi Sad, Serbia
[3] Dr Mirjana Bogavac, Faculty of Medicine
   Clinic of Gynecology and Obstetrics, Hajduk Veljkova 3
   21000 Novi Sad, Serbia
[4] Dr Ljubiša Džambas, Faculty of Medicine
   Department of Pharmacy, Hajduk Veljkova 3
   21000 Novi Sad, Serbia

URTICARIA CAUSED BY
ARCANOBACTERIUM HAEMOLYTICUM. DIAGNOSTIC
AND THERAPEUTIC FAILURES

ABSTRACT: We presented a case of seventeen-year-old girl exhibiting mild symptoms of sore throat, a marked urticarial rash and heavy desquamations of the skin on palms and soles. According to the antibiogram and diagnosis of *Streptococcus* non A non B group obtained in routine laboratory procedure, the patient was treated with penicillin; however, ineffectively. Escalation of urticaria and failure of the initial penicillin therapy shifted the diagnosis towards *exanthema toxoalergicum* and thus to treatment with corticosteroids and antihistamines, yet with no improvement. The culture was repeated with a new specimen. Diagnosis was made according to the specific pattern in a double CAMP test, and confirmed by the ApiCoryne diagnostic set (BioMerieux).

With respect to cultural traits, the isolate mimicked *Streptococcus pyogenes*, yet developing specific pattern in a double CAMP test that directed our diagnosis towards coryneform microorganisms. The diagnosis of *Arcanobacterium haemolyticum* was confirmed with 99.9% probability rate and T = 0.75.

KEY WORDS: *Arcanobacterium haemolyticum*, exanthema, diagnosis, erythromycin therapy

INTRODUCTION

Arcanobacterium haemolyticum (*A. haemolyticum*) is one of the four species of the genus *Arcanobacterium* [R a m o s, 1977] exhibiting all characteri-

stics of irregular non-sporulating Gram-positive rods [H o l t, 1994; F u n k e, 1997].

Man is the primary reservoir and the most frequently affected species, though there are reports of isolates of animal origin [R i c h a r d s o n and S m i t h, 1968; R o b e r t s, 1969; Y o u n a n and D r e s c h e r, 1996; S u - v a j d ž i ć et al., 2002; S u v a j d ž i ć et al., 2002]. Young adults are the most frequently affected population [M i l e r et al., 1986; S l a r r i d g e, 1989; C a r l s o n et al., 1994; N y m a n and B a n c h, 1984], but there are reports of infection in children [K a r p a t h i o s et al., 1992]. In immunocompromi- sed individuals, infections of the upper respiratory tract are mostly induced [M a c L e a n et al., 1946; M a c k e n z i e et al., 1995; C h a l u p a et al., 1995], which may be associated with a rash [B a n c k and N y m a n, 1986] of the scarlatinoform [C a r l s o n et al., 1994] or urticarial type and enlarged lymph nodes, particularly in the neck region [B a n c k and N y m a n, 1984]. Clinical symptoms mimicking rash fevers of viral etiology and undefined aller- gic events often result in overlooking this organism in a suspect diagnosis. Due to its close similarity to the beta-hemolytic species of the genus *Strepto- coccus* and nondescript colonial architecture, it is often misidentified in a rou- tine practice, though its pathogenic potential is well established as early as 1946 [M a c L e a n et al., 1946].

MATERIAL AND METHODS

Study subject

The patient was a seventeen-year-old girl without personal or family history of chronic disease or allergy. She was admitted for examination becau- se of a skin rash in the chest region. The patient was in good general condi- tion, with slightly increased body temperature (37.3°C) and without any su- bjective complaints.

Physical examination revealed a light-pink macular rash, circular in sha- pe, with no skin elevation, with distinct maculae surrounded by unchanged skin. Signs of mild rhinopharyngitis were evident, as well as a slight enlarge- ment of a submental lymph node (about 1 cm in diameter). There were no changes on other organs and organ systems. The presentations were characteri- zed as *Rubella* and symptomatic therapy was recommended.

After 2—3 days, changes of rash appearance and distribution became ob- vious, extending across the body and extremities, being most prominent at the lower abdomen. Efflorescences were maculopapular, slightly elevated from the skin level, red, oval, distinct or convergent (particularly on the extremities), different in size, and with zones of healthy (unchanged) skin amongst them. Pronounced pruritus was present. Body temperature was slightly increased (37.3°C), the pharynx was hyperaemic without enanthema, and the submental lymph node was unchanged with respect to the previous examination.

Laboratory findings showed a slightly increased sedimentation rate, 20 mm/hour. Blood count revealed leucocytosis 15×10^3/l with prevailing neutro-

phyles (87%), fibrinogen 4.0. Billirubin, transaminase and gamma GT values were within the limits of normal ones. Electrophoresis of serum proteins was not performed due to technical reasons.

Complement fixation test (CFT): Adeno 1/16, *Mycoplasma pneumoniae* < 1/4, *Chlamydia trachomatis* (group Ag) < 1/2, Paul Bunell negative

Enzyme Linked Immunosorbent Assy (ELISA) IgM Rubella negative

Pharyngeal swab: *Streptococcus* non A non B group

Recommended therapy — Fenoxymethylpenicillin (Cliacil®).

The therapy applied in the first week was ineffective.

The efflorescences gradually resolved over a period of seven days, but new ones of the same characteristics occurred in other skin areas. Around day 12 after the commencement of the rash, discrete scaling of palms was noted, progressing to massive desquamation of large epidermal areas within 2—3 days. Palms and soles were extremely painful to palpation.

Skin changes were characterized as *exanthema toxoallergicum* by the epidemiologist and dermatologist. The dermatologist suggested antihistaminic therapy with terfenadine (Bronal®) and corticosteroids (Dexason® tb.). The therapy was administered during the second week of illness, without any improvement with respect to skin changes.

In the third week of illness, the rash gradually diminished and desquamation of palms and soles ended. The overall condition of the patient was good, except for the malaise and fatigue. The patient was referred by his physician to the microbiology laboratory of the Department of Pharmacy with the aim of receiving specific therapy. In that respect, the pharyngeal swab was repeated, revealing presence of *Arcanobacterium haemolyticum*. We suggested a therapy with erythromycin, 500 mg, 2 times per day, during 7 days. The therapy was administered in the third week of illness. Improvement occurred 2 days following the administration of erythromycin.

MICROBIOLOGICAL METHODS

The pharyngeal swab was simultaneously inoculated with and without growth lines of *Staphylococcus aureus* (2 plates of each). Subcultures were cultivated on two thioglycolate and one nutrient broth. Thioglycolates were incubated at 37 and 44°C, and the nutrient broth was kept in refrigerator at +4°C. After 48 hours, thioglycolates were subcultured onto three blood agars, which were incubated aerobically, anaerobically and microaerophilically. The nutrient broth was incubated at +4°C during 7 days. Subcultures on blood agar were made daily.

Preparations of primoisolates and subcultures were Gram stained, and the grown colonies examined in catalase, oxidase and esculin tests. "Double CAMP test" (*Rhodococcus*-a *ureus* CAMP at the same blood agar plate) [4, 18] was performed, and possibility of bacterial growth in the presence of bile acids (0.33% cholic and 0.33% monoketocholic acid) was investigated. Ability of agglutination with streptococcal serums in the commercial Slidex Strepto Bio-Merieux set (Bio Merieux, Marcy-l'Etoile, France) was also tested. Final diag-

nosis was performed with the API Coryne (Bio Merieux, Marcy-l'Etoile, France) diagnostic kit, and later evaluated using a software program from the same manufacturer.

RESULTS

In all incubation and temperature conditions, small hemolytic colonies grew on blood agar, which strongly resembled beta haemolytic cocci. However, they did not agglutinate with any of streptococcal diagnostic sera in the SLIDEX Strepto-kit (BioMerieux, Marcy-l'Etoile, France). Further thorough investigation of the subcultures revealed Gram-positive rods in young cultures (up to 18 hours of age), which, with age, changed their morphology towards pleomorphism and polychromasia. The tendency towards Gram-labile, granulated, irregular coccoid form culminated as early as in 24 hours. Oxydase, catalase and esculin tests were negative. In a double CAMP test the isolate produced a strong restriction of haemolysis of *S. aureus* and a marked synergistic haemolysis with *Rhodococcus equi*, with a characteristic "open umbrella" shaped pattern (Figures 1, 2 and 3). Biochemical characteristics are summarized in Table 1.

Fig. 1 — CAMP phenomenon on day 1 of incubation

Fig. 2 — CAMP phenomenon on day 2 of incubation

Fig. 3 — CAMP phenomenon on day 3 of incubation

Note development of the specific pattern of species-specific *A. haemolyticum* in a double CAMP test — synergistic haemolysis with *Rhodococcus equi* ("open umbrella") and restriction of beta-haemolysis of *Staphylococcus aureus*

Key Note:
Right vertical line = *Staphylococcus aureus*
Left vertical line = *Rhodococcus equi*
Top and bottom horizontal lines = *Streptococcus agalactiae*
Two horizontal lines in the middle = *Arcanobacterium haemolyticum*

Tab. 1 — Biochemical characteristics of the investigated *A. haemolyticum* strain. Identification table of % positive reaction after 24ʰ at 35—37°C

| TEST | REACTION | ISOLATE REACTION | |
| | | INVESTIGATED STRAIN | IDENTIFICATION TABLE |
		1	2
NIT	NITrate reduction	0/1	1
PYZ	PYraZinamidase	1/1	98
PyrA	Pyrolydonil Arylamidase	0/1	70
PAL	Alkaline phosphatase	1/1	85
β-GUR	Beta glucuronidase	0/1	36
β-GAL	Beta galactosidase	1/1	89
α-GLU	Alpha glucosidase	0/1	92
β-NAG	N-A-Glucosaminidase	1/1	89
ESC	Esculin	0/1	0
URE	Urea	0/1	0

The result was red using the BioMerieux software program (Bio Merieux, Marcy-l'Etoile, France), being *A. haemolyticum*, with the probability rate of 99.9% and T = 0.75. The only parameter that departed from the identification table was alpha glucosidase negativity, which should have been positive in 92%.

DISCUSSION

A. haemolyticum was isolated in a 17-year-old patient. According to the results of M i l l e r et al. (1986), this organism is mostly isolated in teenagers and younger adults. This was also confirmed by C l a r r i d g e (1989) and C a r l s o n et al. (1994).

The organism was isolated from the pharynx, which was confirmed as the predilection site by several authors [M a c L e a n et al., 1946; M a c k e n z i e, 1995; C h a l u p a et al., 1995].

The rash was the dominant finding, preceding sore throat, rather urticarial then scarlatinoform, and accompanied by pruritus. Skin scaling followed by desquamation of palms and soles was obvious. These changes are not typical for pharyngitis caused by *A. haemolyticum* [W a g n e r, 1991] but there are several descriptions in the literature [M a c k e n z i e, 1995; C h a l u p a et al.,

1995; B a n c h, 1986]. The initial report described one of 12 patients with rash attributed to allergy, diagnosed as exanthema toxoallergicum, same as our patient [M a c L e a n et al., 1946].

A. haemolyticum was isolated in abundance, without presence of other potential pathogenic bacterial agents. Streptococcus-like colonies with coryne-form microscopic appearance were suggestive of *Arcanobacterium / Actino-myces* spp. The diagnosis was made according to the characteristic pattern in the double CAMP test. Haemolysis restriction of *S. auerus* and synergism with the equi factor of *R. equi* is pathognomonic for *A. haemolyticum* [C l a r i d g e and S p i e g e l, 1995; S u v a j d ž i ć et al., 1998], which was confirmed by the APICoryne diagnostic set program (Bio Merieux, Marcy-l'Etoile, France).

By the available methodology, viral agents *Chlamydia trachomatis* and *M. pneumoniae* were excluded, thus we believe *A. haemolyticum* is the causative agent of the described status. The patient was treated with penicillin that, in spite of its good activity *in vitro*, did not result in any improvement. This result is consistent with reports of Banck, Nyman and Osterlund [B a n c k and N y m a n, 1986; N y m a n and B a n c h, 1984; O s t e r l u n g, 1995; N y - m a n and B a n c h, 1990].

CONCLUSION

Prompt etiological diagnosis is of crucial importance for clinicians, protecting patients and physicians from diagnostic and therapeutic failures (in this case the patient could have been spared from cortisone and antihistaminic therapy). In cases of a rash of unclear etiology accompanied by pharyngitis, we suggest excluding the presence of *A. haemolyticum*. In the case that the microbiology laboratory does not have relatively expensive diagnostic sets (BioMerieux or else), diagnosis can be made at the cost of a single blood agar Petri-plate, using characteristic pattern in a double CAMP test, which is species--specific.

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УРТИКА ИЗАЗВАНА *ARCANOBACTERIUM HAEMOLYTICUM*-ОМ. ДИЈАГНОСТИЧКА И ТЕРАПИЈСКА ЛУТАЊА*

Љиљана Ђ. Сувајџић[1], Екатерина А. Мрђа[2], Мирјана А. Богавац[3], Љубиша Д. Џамбас[4]

[1] Медицински факултет, Одсек за фармацију,
Хајдук Вељкова 3, 21000 Нови Сад, Србија
[2] Здравствена установа „Медлаб”, Завод за лабораторијску дијагностику,
Илије Огњеновића 7, 21000 Нови Сад, Србија
[3] Медицински факултет, Клинички центар — Одсек за гинекологију
и акушерство, Хајдук Вељкова 3, 21000 Нови Сад, Србија
[4] Медицински факултет, Клиника за стоматологију,
Хајдук Вељкова 3, 21000 Нови Сад, Србија

Резиме

Arcanobacterium haemolyticum се ретко изолује као узрочник фарингитиса који је у 30% случајева праћен егзантемом. У рутинској пракси обично се превиди јер клинички имитира алергијски, постстрептококни или осип вирусне етиологије. Приказујемо случај седамнаестогодишње девојке која је имала благе симптоме упале грла праћене наглашеним уртикаријалним рашом са последичном десквамацијом коже стопала и дланова. Водећи се резултатом антибиограма и бактериолошком дијагнозом *Streptococcus* nonA nonB групе пацијент је третиран пеницилином али безуспешно. Ескалација уртике након увођења пеницилина завела је клиничара ка дијагнози *exanthema toxoalergicum*. Стога је настављен третман антихистаминицима и кортикостероидима такође безуспешно. Поновно узет брис је бактериолошки обрађен у циљу искључивања коринеформних бактерија. На основу специфичног резултата у двоструком CAMP тесту посумњано је на *Arcanobacterium haemolyticum*, а дијагноза је потврђена дијагностичким сетом ApiCoryne diagnostic set. Софтверски програм истог произвођача дијагнозу *Arcanobacterium haemolyticum* је оценио као одличну, са вероватноћом идентитета 99,9% уз степен сигурности T = 0.75. Оваква дијагноза сугерисала је еритромицинску терапију — 500 mg 2 пута дневно у трајању од 7 дана што је довело до потпуног излечења пацијента. Сходно томе у случајевима раша нејасне етиологије удружене са фарингитисом сугеришемо искључивање *Arcanobacterium haemolyticum*. Прецизна микробиолошка дијагноза доступна је свакој рутинској лабораторији уколико се уместо обичног CAMP теста у случају бета хемолитичких колонија стрептококног изгледа користи двоструки CAMP тест.

D r a g a n V. D o d e r[1]
R a d o s l a v a Ž. D o d e r[2]

[1] Vojvodina Province Sports Institute
   Masarikova 25, 21000 Novi Sad, Serbia
[2] Clinic for infectionses diseases
   Hajduk Veljkova 1—9, 21000 Novi Sad, Serbia

EFFECT OF ANTHROPOLOGICAL CHARACTERISTICS ON THE EFFICIENCY OF EXECUTION OF FORWARD KICK

ABSTRACT: A sample of eighty-two karatists at the ages from ten to fourteen has been analyzed for a system of 25 variables (12 morphological, 12 basic motoric variables and 1 specific motoric variable) with the aim of establishing the effect of prediction system of morphological variables and a system of basic motoric variables on the criterion variable, i.e., the direct forward kick — mae geri.

The obtained results showed that the system of morphological variables had a statistically significant effect on the execution of direct forward kick. Among the individual variables in the regression analysis, body weight had the largest effect. The stepwise method showed that body height and weight had highest prediction values. Young karatists of high body height, with long extremities and increased weight, had better results in the execution of direct forward kick.

The investigation of basic motoric variables used in the regression and stepwise analyses indicated that the endurance in half-squat with weight and standing jump had statistically significant effects on the efficiency of direct forward kick. Thus it was concluded that the speed of forward kick depends on the explosive and static strength of legs.

KEY WORDS: karate, morphological characteristics, motoric abilities, effect

INTRODUCTION

During the conceptualization and operationalization of this research, it was assumed that the system of prediction of morphological and basic motoric variables has a significant effect on some specific motoric structures (technical elements of karate) of more complex type, such as the criterion variable.

The aim of this researh was to establish the influence of prediction system of morphological variables on the criterion variable — direct forward kick (mae geri).

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SAMPLE AND METHODS

The sample of eighty-two karatists at the ages from ten to fourteen was analyzed for the system of 25 variables: 12 morphological ones, 12 basic motoric variables and 1 specifically motoric variable. The representative sample of karatist used in this research consisted of eighty-two male members of eighteen karate clubs from the Vojvodina Province (Serbia and Montenegro).

Instruments

The estimation of morphological characteristics was based on the following measurements:

Longitudinal measurements of the skeleton: body height (HEIGHT), leg length (LLENG) and arm length (ALENG).

Transversal measurements of the skeleton: shoulder breadth (BRESH), pelvis breadth (BREPEL), and the wrist diameter of (DIAWR).

Body mass and volume: middle bust measurement (MIDBUST), forearm measurement (FOREARM), mass of body (BODYMASS).

Subcutaneous fatty tissue: skin fold of the upper arm (SUPARM), skin fold of the abdomen (SABDOM), skin fold of the back (SBACK).

While choosing anthropometric measures, attention was paid to select those measures which will best represent the above four characteristics, as well as those which have the best metric characteristics. Each of the abovementioned morphological characteristics was covered by three measures. After a series of tests, it was establish thet neither the number nor the structure of morphological characteristic change under the influence of the number of anthropometric variables, which means that the morphological dimensions (longitudinal dimension of the skeleton, transversal dimension of the skeleton, body volume and mass and subcutaneous fatty tissue) have fully consistent values (M o m i - r o v i ć, 1969, K u r e l i ć et al., 1975, S t o j a n o v i ć et al., 1975).

Following tests were applied for the estimation of basic motoric abilities:

1. The mechanism for movement structuring: agility in the air (AIRAG), arm tapping (ARMTAP), leg tapping (LEGTAP).

2. The mechanism for tonus and synergy regulation: forward bend on a bench (FORB), one-leg standing along the balance beam (LEGST) and step forward with a baton (STEPFB).

3. The mechanism for regulation of excitation duration: torso lifting for 30 seconds (TORLIF), pushups on parallel bars (PUSH-UPS) and endurance in half-squat with weight (HSQEND).

4. The mechanism for regulation of excitation intensity: standing jump (LOJP), triple standing jump (TRIJP), 20m run from the high start (RUN20m).

The factors and metric characteristics of the selected motoric variables have been tested in a large number of researches (K u r e l i ć et al., 1975, G r e d e l j et al., 1975). This model was chosen because it provided the most

relevant information which are connected with the functional structure of motoric abilities.

The criterion variable for the estimation of specific motoric abilities was:

Direct forward kick by the tip of the sole — mae geri (KICKMG)

When choosing the specific motoric variable, our choice went in favor a variable which would best represent the karate leg techniques and which showed good metric characteristics in previous researches (K a j č e v s k i, 1976, Z u l i ć et al., 1985, M i l o š e v i ć and Z u l i ć, 1988a, 1988b, J o v a n o - v i ć, 1988, A r l o v, 1993, M u d r i ć, 1994). The quoted references were used when testing specific speed abilites in the methodological laboratory of the Faculty of PE in Belgrade.

Procedures

The measurements were conducted in the gym of the Center for Physical Education of Students of the Faculty of PE in Novi Sad. During the measurements, the hall was well lit and the temperature was between 18° and 22°C.

Description of anthropometric measurements:

The selection and marking of measuring spots on the body and anthropometric measurements were carried out by the method of International Biological Programme (IBP). The measurements were conducted in the morning, with calibrated instruments. The subjects were barefoot and in pants.

Description of the measurements of basic motoric abilities:

The ground for testing the basic motoric abilities was set up before the official beginning of the measurements. All subjects were measured by the same group of measurers, who had underwent a thorough training. The schedule of basic motoric tests was same for all subjects. The tests were so arranged that there was no influence of fatigue after harder tests on the results of subsequent tests.

The following instruments and accessories were used in the measurement program: stopwatches, tatami mattresses, hand and foot tapping boards, a stool, a low balance beam, a flat bench, a wooden measuring rod, a baton, a tape measure, parallel bars, weights, a protractor, a spring board, a steel measuring tape, magnesium, a 10-meter rubber floor mat and stands.

Description of the measurement of specific motoric abilities:

During the measurements, a single subject was in the test room with three technicians. One was in charge of a contact focuser, another of a computerized timer and the third wrote down the results. The subjects wore kimonos and they were barefoot. Before measuring, the subjects were familiarized with the technique they were expected to perform. Starting line and the height of contact area were precisely determined before the execution.

The subject was to assume a fighting stance (fudo dachi) at the distance of one meter away from the arm focuser. At the moment of the kick, the body is on one leg, bent in the knee for balance, while the arms are on guard with head and torso in vertical position. When signal is sounded, the kisk is executed with the back leg, the tip of the sole hitting the focuser set at the he-

ight of 80 cm from the ground. The best time from three attempts is recorded, with the accuracy of measurement of 1/100seconds.

Data processing

The following central and dispersion parameters were estimated for each of the tested variables: arithmetic mean (X), standard deviation, (S), standard error of arithmetic mean (SX), minimal value (Min), maximal value (Max), variation breadth (VB), skewness (Sk) and kurtosis (Ku).

Regression analysis was used to estimate the effect of the system of predictor variables on individual criterion variables.

A stepwise method was applied within the regression analysis to estimate the prediction value (prognostic worth) of reduced predictor variables.

The obtained data were processed by the statistical packages STATISTICA (6.0) and SPSS (11.5).

RESULTS AND DISCUSSION

Basic central and dispersion parameters of the variables and their discriminative level

It is evident from Table 1 that most of the applied morphological variables did not deviate considerably from the normal distribution. Exceptions were registered for the measures of skin fold of the back (Sk = 1.23), skin fold of the abdomen (Sk = 1.67) and pelvis breadth (Sk = 1.23) whose positive measures of asymmetry showed that the young karatists had a somewhat wider pelvis and an increased amount of subcutaneous fatty tissue. Distribution curves of all variables were either mesokurtic or platykurtic, except those for the skin fold of the back (Ku = 6.58) and pelvis breadth (Ku = 5.39) which were leptokurtic.

Tab. 1 — Central and dispersion parameters of the morphological variables

Variables	X	Min	Max	VŠ	S	SX	SK	Ku
HEIGHT	149.88	120.90	182.40	61.50	13.16	1.45	.21*	−.43
LLENG	86.05	71.70	104.30	32.60	7.72	.85	.16*	−.67
ALENG	63.19	49.60	79.10	29.50	6.26	.69	.28*	−.33
BRESH	31.98	25.10	41.50	16.40	3.17	.35	.21*	.08
BREPEL	23.23	15.90	36.40	20.50	2.77	.30	1.23	5.39
DIAWR	4.80	3.90	5.90	2.00	.46	.05	.11*	−.52
MIDBUST	70.35	56.50	91.50	35.00	7.96	.87	.38*	−.43
FOREARM	21.05	17.10	29.20	12.10	2.26	.25	.66*	1.10
WEIGHT	39.68	20.00	72.00	52.00	11.05	1.22	.53*	−.07
SUPARM	10.64	1.60	23.60	22.00	4.35	.48	.89*	.57
SABDOM	10.83	2.40	40.00	37.60	8.53	.94	1.67	2.38
SBACK	9.50	4.00	34.80	30.80	5.41	.59	2.35	6.58

Most of the tested basic motoric variables did not deviate from normal distribution (Table 2). That shows that the selected tests were sufficiently discriminative, except for the variables of flamingo balance (Sk = 2.25), pushups on parallel bars and the depth of forward bend on the bench (Sk = 1.17), in which the distribution of results was assymetric because poor results were more numerous. The distribution curve for the first variable was leptokurtic (Kn = 7.69); the curves for the other two variables were mesokurtic and platykurtic, respectively.

Tab. 2 — Central and dispersion parameters of the basic motoric variables

Variables	X	Min	Max	VŠ	S	S8	SK	Ku
AIRAG	15.30	11.80	20.00	8.20	1.79	.19	.55*	.49
ARMTAP	41.37	24.00	55.00	31.00	6.70	.74	.09*	−.16
LEGTAP	53.87	32.00	66.00	34.00	5.61	.61	−.55*	1.80
FORB	42.95	17.00	53.00	36.00	6.61	.73	−1.17	2.57
LEGST	14.31	1.70	59.20	57.50	9.57	1.05	2.25	7.69
STEPFB	5.99	2.30	8.60	6.30	1.19	.13	−.58*	.78
TORLIF	23.12	15.00	34.00	19.00	3.31	.36	−.08*	.90
PSHUPS	1.62	.00	8.00	8.00	2.14	.23	1.64	2.07
HSQEND	4.69	4.00	11.40	7.40	2.05	2.26	.73*	1.08
LOJP	161.13	105.00	240.00	135.00	25.61	2.82	.11*	−.01
TRIJP	502.20	313.00	700.00	387.00	71.15	7.85	.32*	.27
RUN20M	3.83	3.10	4.80	1.70	.35	.03	.18*	−.15

The distribution of results for the specific motoric abilities did not deviate significantly from normal distribution (Table 3), which showed that the applied test was satisfactorily discriminative.

Tab. 3 — Central and dispersion parameters of the specific motoric variable

Variables	X	Min	Max	VŠ	S	SX	SK	Ku
DIRKICK	.84	.63	1.06	.50	.11	.11	−.19*	−.49

Effect of the system of morphological variables
on the criterion variable — mae geri

The obtained values presented in Table 4 showed that the multiple correlation was .60 (Ro = .60) and that the applied prediction system of morphologic variables had a statistically significant effect on the criterion variable DIRKICK (mae geri) at the level of statistical significance of 0.00 (p = .00). As the square of the multiple correlation was .36 (Ro2 = .36), it means that 36% of the total variance could be used to successfully predict the criterion variable on the basis of the applied system of morphological variables.

Among the individual values of statistical parameters within the regression analysis of the applied prediction system of morphologic variables, only body weight exerted an effect on the criterion variable that was statistically significant at 0.01 (p = .01).

Therefore, it may be stated that the subjects with larger body weight achieved better results in the execution of the direct forward kick (better time of executing the kick).

Tab. 4 — Effect of predicting morphological variables on the criterion variable DIRKICK

Variables	b	E(b)	T	p
HEIGHT	−.46	.45	−1.03	.30
LLENG	−.19	.30	−.65	.51
ALENG	−.16	.24	−.66	.50
BRESH	−.25	.20	−1.23	.22
BREPEL	.03	.17	.21	.82
DIAWR	−.16	.24	−.65	.51
MIDBUST	−.14	.25	−.55	.58
FOREARM	−.22	.27	−.81	.41
WEIGHT	1.11	.46	2.41	.01*
SUPARM	.17	.12	1.39	.16
SABDOM	.09	.22	.44	.65
SBACK	−.26	.18	−1.41	.16

$Ro^2 = .36$ $Ro = .60$ $F = 3.36$ $p = .00$

The stepwise analysis (Table 5) showed that the obtained value of multiple correlation was .59 (Ro = .59) and that a reduced system of 6 morphological variables had a statistically significant influence on the execution of mae geri at the level of statistical significance of 0.00 (p = .00). As the square of the multiple correlation was .35 ($Ro2 = .35$), it means that 35% of the total variance could be used to successfully predict the criterion variable on the basis of the reduced system of 6 morphologic variables.

Body height had the highest prediction value, explaining 22% of the total variability at the level of statistical significance of 0.00 (p = .00). Body weight was in the second place, explaining 6% of the variability at the level of statistical significance of 0.00 (p = .00).

Thus it may be stated that the young karatists with higher body height, therefore having longer extremities and increased body weight, had better results in performing the direct forward kick, primarily on account on increased voluminousness of the body and the long and elastic musculature.

Tab. 5 — Prediction values of the morphological variables

Variables	Ro	Ro2	Ro2(p)	F	p
HEIGHT	.47	.22	.22	23.57	.00*
WEIGHT	.54	.29	.06	7.36	.00*
BRESH	.55	.31	.01	1.91	.17
SUPARM	.56	.32	.01	1.62	.20
SBACK	.58	.34	.01	2.05	.15
DIAWR	.59	.34	.00	1.03	.31

$Ro^2 = .35$ $Ro = .59$ $F = 6.77$ $p = .00$

*Effect of the system of basic motoric variables
on the criterion variable — mae geri*

The system of predicting basic motoric variables (Table 6) had a statistically significant effect on the criterion variable DIRKICK (mae geri) at the level .00 (p = .00), with the multiple correlation value of .69 (Ro = .69). As the square of the multiple correlation was .48 (Ro2 = .48), it means that 48% of the total variance could explain the predicting value of the basic motoric variables for the criterion variable — mae geri.

The analysis of individual predicting basic motoric variables indicated that the endurance in half-squat with weight and standing jump had statistically significant influences on the criterion variable (mae geri) at the level of 0.05 (p = .05)

Thus it could be concluded that a fast and explosive execution of mae geri depends on the explosive and static power of legs.

Tab. 6 — Effect of predicting basic motoric variables on the criterion variable DIRKICK

Variables	b	E(b)	t	p
AIRAG	.07	.11	.69	.49
ARMTAP	.05	.11	.43	.66
LEGTAP	.04	.11	.41	.68
FORB	−.10	.11	−.91	.36
LEGST	.02	.10	.20	.83
STEPFB	.04	.10	.39	.69
TORLIF	−.18	.11	−1.58	.11
PSHUPS	−.16	.11	−1.44	.15
HSQEND	.37	.11	3.27	.00*
LOJP	−.29	.14	−1.99	.05*
TRIJP	−.21	.15	−1.45	.15
RUN20M	−.01	.13	−.08	.93

Ro^2 = .48 Ro = .69 F = 5.41 p = .00

The stepwise method (Table 7) showed that the multiple correlation was .68 (Ro2 = .68) and that the reduced system of 6 basic motoric variables had a statistically significant influence on the criterion variable (mae geri) at the level of statistical significance of .00 (p = .00). As the square of the multiple correlation was .47 (p = .47), it means that 47% of the total variance could be used to successfully predict the criterion variable on the basis of the system of basic motoric variables.

Standing jump had the highest prediction value at the level of the statistical significance of .00 (p = .00), explaining as much as 33% of the total variance. Endurance in half-squat with weight and triple standing jump were in the second and third place with 6% (p = .00) and only 3% (p = .02), respectively.

Considering the above, it could be concluded that the static power of legs had a primary role and the explosive power of legs had a second-important role for the speed of executing the forward kick. Static power is responsible for stabilization of the leg which on which we lean and for lifting in starting posi-

tion the knee of the leg which does the kick. Secondly, success of the kick depends on the contractile strength of muscles of the lower stomach and the explosive power of the muscles of the hip, knee and ankle which are responsible for the execution and return of the leg which does the kick.

Tab. 7 — Prediction values of the basic motoric variables

Variable	Ro	Ro2	Ro2(p)	F	p
LOJP	.58	.33	.33	41.01	.00*
HSQEND	.63	.40	.06	8.14	.00*
TRIJP	.66	.43	.03	5.27	.02*
TORLIF	.67	.45	.01	2.48	.11
PSHUPS	.68	.46	.00	1.25	.26
FORB	.68	.47	.01	1.46	.23

Ro^2 = .47 Ro = .68 F = 11.32 p = .00

CONCLUSION

The sample of 82 karatists at the ages from ten to fourteen were analyzed for the system of 25 variables with the aim of establishing the effect of the prediction system of morphological variables and the system of basic motoric variables on the criterion variable, i.e., the direct forward kick — mae geri.

The obtained results showed that the system of morphological and motoric variables have a statistically significant effect on the execution direct forward kick. Among the individual morphological variables, body height and weight had the largest influence, and among the motoric variables those were the endurance in half-squat with weight and standing jump. It may be concluded that young karatists with high body height, long extremities and increased mass (weight), supported by strong static power and explosive strength in legs, showed better results in executing the direct forward kick.

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УТИЦАЈ АНТРОПОЛОШКИХ КАРАКТЕРИСТИКА НА УСПЕШНОСТ ИЗВОЂЕЊА УДАРЦА НОГОМ ПРЕМА НАПРЕД

Драган В. Додер[1], Радослав Ж. Додер[2]
[1] Покрајински завод за спорт, Масарикова 25, 21000 Нови Сад, Србија
[2] Клиника за инфективне болести, Хајдук Вељкова 1—9,
21000 Нови Сад, Србија

Резиме

На узорку од 82 каратиста, узраста од 10 до 14 година, био је примењен систем од укупно 25 варијабли (12 морфолошких, 12 базично моторичких и 1 специфично моторичка варијабла) са циљем да се утврди утицај предикторског си-

стема морфолошких и система базично моторичких варијабли на критеријумску варијаблу директни ударац ногом према напред — mae geri.

Резултати указују да систем морфолошких варијабли има статистички значајан утицај на извођење директног ударца ногом према напред. Од појединачних вредности у оквиру регресионе анализе највећи утицај има тежина тела. Stepwise-методом је утврђено да највећу предикторску вредност имају висина тела и тежина тела. Млади каратисти веће телесне висине, а тиме и дужим екстремитетима и повећаном масом (тежином) имали су боље резултате у извођењу директног ударца ногом према напред.

Анализом базично моторичких варијабли утврђено је да статистички значајне утицаје на извођење директног ударца ногом према напред, у оквиру регресионе и stepwise-анализе, имају издржај у получучњу с оптерећењем и скок удаљ с места. На основу тога се може закључити да брзина извођења ударца ногом према напред зависи од експлозивне и статичке снаге ногу.

E s t e r   J .   P o p o v i ć
O l i v e r a   N .   B j e l i ć - Č a b r i l o
K r i s t i n a   R .   T e p a v a c

Department of Biology and Ecology
Faculty of Natural Sciences, Novi Sad
Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

THE FAUNA OF SMALL MAMMALS IN THE VICINITY OF TEMERIN (THE VOJVODINA PROVINCE)

ABSTRACT: The samples collected by traps in the vicinity of Temerin and the analysis of pellets of the long-eared owl (*Asio otus*) and the barn owl (*Tyto alba*) served as a basis for the determination of 409 individuals of small mammals of the orders *Insectivora* and *Rodentia*. A total of 13 species from the families *Soricidae* (6), *Muridae* (4) and *Arvicolidae* (3) was recorded. The representatives of the species *Apodemus sylvaticus* prevailed in the sample obtained by traps while the pellet analysis showed the domination of *Microtus arvalis* in the owl diet. The presence of five of the total of thirteen species found by the pellet analysis was confirmed by means of traps.

KEY WORDS: pellet analysis, small mammals, Temerin, traps

INTRODUCTION

Long-term investigations of certain types of agrobiocoenoses show that the field vole (*Microtus arvalis*) and the hamster (*Cricetus cricetus*) are the most adaptable rodent species (M i k e š   and   H a b i j a n - M i k e š, 1986). The adaptation strategy of small mammals relies primarily on their biological characteristics such as a high capacity to produce offspring and coincidence of periods of animal activity with the phenology of crops that ensure shelter and their population dynamics. One of the most important traits of the small mammal populations are cyclic oscillations of their numbers where maximum values are recorded each 3—5 years that rely upon both the species itself and the actual ecological conditions. In the overpopulation periods, these species are recognized as serious pests. The absence of their predators, i.e., birds of pray (owls and daylight birds) and carnivorous mammals (wild cats, skunks, ermines) also contributes to their increased high numbers. The owls adapted to feed on small mammals significantly regulate their numbers. A specificity of owl diet is the formation of pellets (indigestible parts such as fur or feathers

regurgitated with bones). Therefore, the analysis of pellet content gives relevant data on the diversity, distribution, numbers, and behavior of pray.

The aim of our investigation was to study a season-dependent diversity of small mammals occurring in a typical agrobiocoenosis in the vicinity of Temerin (the Vojvodina Province) by analyzing the pellets of two species of owls (*Asio otus* and *Tyto alba*).

MATERIAL AND METHODS

The study of the fauna of small mammals was conducted in the period March 2000—October 2001. Two methods were employed, i.e., traps and pellet analysis. To obtain accurate values, both methods were applied concurrently at the investigated sites, through the vegetation seasons.

Traps

A standard linear method with traps was applied in a clover field (100 x 100 m) surrounded by corn, wheat and soybean. Sampling procedure was repeated 20 times.

Pellet analysis

To determine the diversity of small mammals, the pellets of two owl species, the small-eared owl (*Asio otus* L. 1758) and the barn owl (*Tyto alba* S c o p. 1769) were analyzed. Pellets were collected at two sites located at the ends of the site with traps. In 15 repeated samplings, a total of 173 pellets were collected of which 93 belonged to the long-eared owl and 80 to the barn owl. The analysis included only intact pellets while the indigestible parts of pellet remnants were also determined and listed in the faunal list (S c h m i d t, 1967).

RESULTS

Of the total of 409 representatives of small mammals determined, 347 individuals were recorded by analyzing owl pellets and 62 were found in traps. They belonged to two orders, *Insectivora* (69 individuals making 16.87%) and *Rodentia* (340 individuals making 83.13%), three families (*Soricidae, Muridae* and *Arvicolidae*), and eight genera (*Neomys, Sorex, Crocidura, Pitymys, Microtus, Apodemus, Mus* and *Rattus*). By combining the two methods we recorded the occurrence of a total of 13 species. Five species were recorded by using traps (*Crocidura suaveolens, Microtus arvalis, Apodemus sylvaticus, A. agrarius* and *Mus musculus*) while all 13 species were recorded by the pellet analysis. In addition to the above, the owl diet included also the species *Crocidura leucodon, Sorex araneus, S. minutus, Neomys fodiens* and *N. anomalus*

(shrews) as well as *Microtus agrestis* and *Pitymys subterraneus* (voles) and *Rattus* sp. (Tables 1 and 2).

Tab. 1 — Material collected by using traps at clover fiels

| SPECIES | VEGETATION SEASON | | | | | | | | TOTAL | |
| | SPRING | | SUMMER | | AUTUMN | | WINTER | | | |
	n	%	n	%	n	%	n	%	n	%
Crocidura suaveolens	1	2.27	/	/	/	/	/	/	1	1.61
Apodemus sylvaticus	31	70.45	6	50.00	3	75.00	/	/	40	64.52
Apodemus agrarius	4	9.09	1	8.33	/	/	/	/	5	8.06
Mus musculus	7	15.92	5	41.67	1	25.00	2	100.00	15	24.20
Microtus arvalis	1	2.27	/	/	/	/	/	/	1	1.61
TOTAL	44	70.97	12	19.35	4	6.45	2	3.23	62	100.00

Tab. 2 — Material collected from pellets of species *Asio otus* and *Tyto alba* in the vicinity of Temerin

| PREY SPECIES | *Asio otus* | | *Tyto alba* | | TOTAL | |
	n	%	n	%	n	%
Apodemus sylvaticus	37	23.87	15	7.69	52	14.98
Apodemus agrarius	6	3.87	7	3.59	13	3.75
Mus musculus	14	9.03	15	7.69	29	8.35
Microtus arvalis	80	51.61	58	29.74	138	39.77
Microtus agrestis	—	—	1	0.51	1	0.29
Pitymys subterraneus	8	5.16	16	8.21	24	6.91
Crocidura leucodon	—	—	13	6.67	13	3.75
Crocidura suaveolens	3	1.93	29	14.87	32	9.22
Sorex araneus	1	0.65	17	8.72	18	5.19
Sorex minutus	1	0.65	2	1.03	3	0.86
Neomys fodiens	—	—	1	0.51	1	0.29
Neomys anomalus	—	—	1	0.51	1	0.29
Rattus sp.	1	0.65	11	5.64	12	3.46
Apodemus sp.	4	2.58	3	1.54	7	2.02
Microtus sp.	—	—	3	1.54	3	0.86
TOTAL	155	100.00	192	100.00	347	100.00

The analysis of the diet results showed clearly that the barn owl (*Tyto alba*) had a greater diet diversity, regarding prey type and amount, than the long-eared owl (*Asio otus*). The occurrence of the field vole (*Microtus*) and the water shrew (*Neomys*) in the owl diet showed that the hunting terrain, in addition to arable land, included also the wetland intersected by canals located in the vicinity of the Temerin site. The analysis of pellets of the long-eared owl showed the occurrence of 155 prey individuals (1.6 ind. on the average) vs. 195 in pellets of the barn owl (2.4 ind. on the average) (Table 2).

In respect of the abundance and percentages of small mammals, the family *Muridae* (96.78%) with the representative *Apodemus sylvaticus* (40 ind. making 64.52%) was dominant in the trap samples while the family *Arvicolidae* (56.77% and 40.63%) with the representative *Microtus arvalis* (52.6% and 29.74%) was dominant in the pellet samples. In addition to voles, the diet of the long-eared owl also included considerable amounts of mice (40%) while the diet of the barn owl included shrews (32.81%); therefore, the owl species escaped the competition for food (Figures 1, 2, 3; Tables 1, 2).

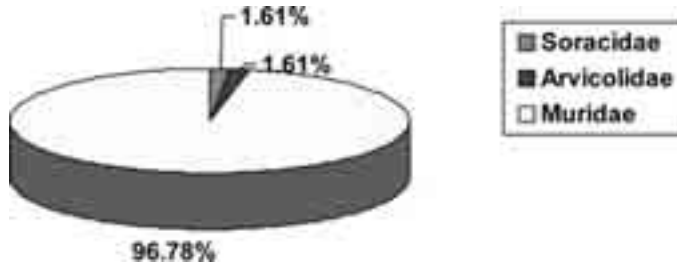Fig. 1: Percentages of families of small mammals caught by traps

Fig. 2: Percentages of small mammals in the pallet semple of the Long-Eard Owl (*Asio otus*)
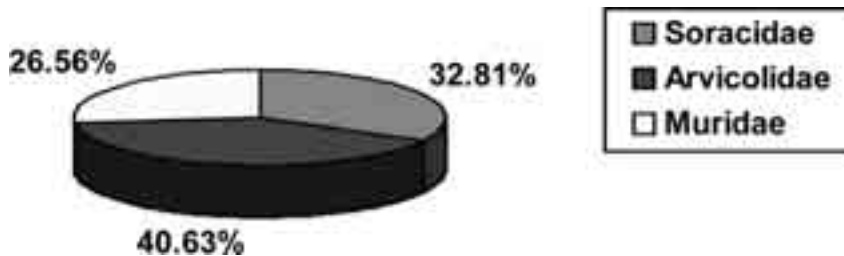

Fig. 3 — Percentages of families of small mammals in the pallet semple of the Bran Owl (*Tyto alba*)

DISCUSSION

Not only the biology but, also the living habits and behavior of small mammals contribute to the complex nature of this group of vertebrates. There-

fore, the method of traps is not relevant enough to give comprehensive data on their fauna, distribution, and abundance as well as the role of a species in a given biocoenosis. This method offers no reliable data on their diversity, particularly in years when the numbers of their populations are low (M i k e š and H a b i j a n - M i k e š, 1989).

With the exception of a single specimen of the garden shrew (*Sorex*) and one field vole (*Microtus*), the field mouse was dominant (64.52%) in the built traps. The percentage of the striped mouse was low (8.06%) while the percentage of the house mouse was considerable (24.2%). The latter is a synantropic species occurring both in urban and agricultural areas. Shrew and vole species that were absent from traps obviously inhabit more humid habitats, i.e., they are rare in agroecosystems. In respect of diet, they may be herbivorous or insectivorous (shrews). The bait used in our investigation was highly attractive for omnivorous species like the representatives of the family *Muridae*.

Complex theriological investigations into the agroecosystems of the Vojvodina Province showed that *Apodemus sylvaticus* was not only present but was also the dominant species at the investigated sites. House mouse (*Mus*) was also recorded in traps but in small numbers while remarkable cyclic fluctuations in population number of *Microtus arvalis* were evident (M i k e š and H a b i j a n - M i k e š, 1986). In our analysis this species was dominant in the pellet samples of both the long-eared owl and the barn owl, with 138 individuals (39.77%) in the total sample. Its high population density and the formation of colonies in agrobiocoenoses, its activity period and rather slow movements wnen compared with *Mus* make the field vole an easy prey for the owls.

The long-eared owl was found at forest edges in the close vicinity of arable lands, whereas the barn owl prevailed in urban habitats. Their common feature was predation in open terrains. The prey weight affects both owl species more than prey type. In other words, prey weight less than 100 g attracts the long-eared owl while that between 5 and 30 g the barn owl (M i k k o l a, 1983). Also, differences have been reported in energy requirement per day, resulting from different body weight of the owls. Consequently, the barn owl catches between 70 and 140 g of prey per day that contributes to a greater variety of small mammals in its diet (M i k k o l a, 1983). Our results corresponding with published data show that the barn owl diet included 13 species (192 individuals) while the diet of the long-eared owl included 9 species (155 individuals). In addition to the field vole, shrews were recorded in the diet of the barn owl, particularly the garden shrew (14.87%), while mice (*Apodemus*) prevailed in the diet of the long-eared owl (23.87%, Table 2).

The results obtained by building traps and collecting and analyzing pellets of the long-eared owl and the barn owl emphasize the benefits of simultaneous application of both methods in faunal and ecological investigations of small mammals, to obtain comprehensive and reliable data on their diversity, population density, and distribution.

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ФАУНА СИТНИХ СИСАРА ОКОЛИНЕ ТЕМЕРИНА

Естер Ј. Поповић, Оливера Н. Бјелић-Чабрило, Кристина Р. Тепавац
Департман за биологију и екологију, Природно-математички факултет
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Србија

Резиме

Фауне ситних сисара истраживане су у периоду од марта 2000. до октобра 2001. године на подручју Темерина. Коришћене су 2 методе: метода ловних клопки преклопног типа и анализа гвалица сова: *Asio otus* и *Tyto alba*. Детерминисано је укупно 409 примерака ситних сисара: 347 јединки из гвалица и 62 примерка из ловних клопки. Ситни сисари су били заступљени са 13 врста припадника породица: *Soricidae, Muridae* и *Arvicolidae*. Пет врста је регистровано методом ловних клопки, а свих 13 анализом гвалица сова. Врста *Apodemus sylvaticus* је доминирала у узорку из ловних клопки, а *Microtus arvalis* у гвалицама сова. Констатована је већа разноврсност плена кукувије, како у односу на врсте тако и у односу на квантитативни удео. Поред волухарице, у оброцима хране мале ушаре запажено је значајно учешће мишева, а код кукувије — ровчица.

Aleksandra D. Tišma

Netherlands Institute for Spatial Research
Willem Witsenplein 6, 2596 BK The Hague, Netherlands

EVALUATION OF A PUBLIC PARTICIPATION GEOGRAPHIC INFORMATION SYSTEM

ABSTRACT: Evaluating public participation geographic information systems (PP GIS) is essential to make improvements and generate standard practices for future projects, but it is probably the most neglected activity of the PP GIS development cycle. This research makes a contribution to the scarce information available about the usefulness of these systems in planning practice. The research examined the use of the PP GIS which was developed during last five years under the sponsorship of the Dutch Ministry of Spatial Planning, Social Housing and Environment, called "The New Map of the Netherlands".

KEY WORDS: The New Map of the Netherlands, public participation GIS, evaluation

INTRODUCTION

Being the latest member of — in the order of historical appearance — Decision Support Systems (DSS), Spatial Decision Support Systems (SDSS) and Planning Support Systems (PSS), Public Participation Geographic Information Systems[1] (PP GIS) seems to have inherited some of the problem points of the other members: a complex structure which causes low acceptance in practice and an *ad hoc* approach to development and evaluation which is reflected in repeated mistakes.

There are many definitions of PSS, the newest ones to be found in Klosterman's essay on PSS (Environment and Planning B, 2005). But, regardless of the definition, the initial goal of all these systems is to help planners bring decisions in a rational way, supported by arguments, based on proper and accurate information and an awareness of consequences these decisions might have. In addition to these goals, PP GIS has an even more ambitious goal — to involve citizens in the decision making process.

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[1] In this article PP GIS will be considered as one of the versions of PSS, hence some remarks that are in the literature related to PSS are considered relevant for PP GIS as well.

But does this happen? For most citizens the personal benefit of getting involved in planning activities and learning how to use a public participatory GIS application is usually little and the cost of participation is rather high. The cost of participation includes the cost of informing oneself about the form of participation, planned activities and learning how to use a public participatory GIS application. Besides, in most of the cases it is more an academic exercise and the citizens cannot really influence the final planning decisions. In such cases, citizens decide to ignore the possibility of involvement and participation. One of the explanations for this situation is the phenomenon of rational ignorance. According to rational choice theory, ignorance about an issue is said to be rational when the cost of educating oneself about the issue sufficiently to make an informed decision can outweigh any potential benefit one could reasonably expect to gain from that decision, and so it would be irrational to waste time doing so (K r e k, 2005).

In the last few years, voluminous scientific literature emerged in the field of PP GIS, discussing the technical and social issues of such applications. This research field became quite popular within the GIS science community. However, the practical side of this research shows a different picture. Namely, it is difficult to find good, operational, practical examples of such PP GIS applications (K r e k, 2005).

The biggest problem in this case seems to be how to develop a system that would be as good for professionals as for non-professionals and easy to use so it would be adopted by the broad public.

In the last two decades, with the rapid development of computer technology and Internet, numerous researchers and practitioners accepted the challenge to develop PSS for many different purposes. But once developed, most of the systems were not evaluated, as evaluation is one of the most neglected steps in the PSS development process. There are several reasons for insufficient evaluation of PSS and low dissemination of evaluation results. Some of them apply to PP GIS as well. No standard evaluation methods are available to evaluate PSS and in the case of PP GIS the situation is the same. The question is whether it would be possible to develop such a method because the variety of PP GIS applications does not permit a single methodology to serve all purposes (A l e x a n d e r, 1996). In this situation it is also difficult to compare the results of different evaluation methods even when an evaluation is made. Another problem with evaluation follows from this — it costs not only time to make the evaluation but also time to prepare the methodology for it. In most cases time for evaluation is short and usually there will be little room for a true or quasi-experimental setup, due to the need for a control group (B a - t e n b u r g and B o n g e r s, 2001). It is clear that this requires time as well as substantial resources, which is another problem. Evaluations are therefore made rather *ad hoc*, without sufficient attention to sound theoretical grounding that covers the wide range of relevant factors. V o n k et al. (2005) argue that as a result of these difficulties evaluation results are not widely disseminated. Therefore it often happens that the same mistakes are repeated and incorporated in PSS and PP GIS systems.

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This research is aimed at upgrading the knowledge about the implementation of the PSS and in this case PP GIS in the planning practice. The research examined the use of the PP GIS which was developed in the last five years under the sponsorship of the Dutch Ministry of Spatial Planning, Social Housing and Environment, called "The New Map of the Netherlands".

THE NEW MAP OF THE NETHERLANDS

The interactive map of the "New Map of the Netherlands" is a website with a continuously updated overview of spatial plans that are being produced in the Netherlands. The website was developed with the goal of increasing the transparency of spatial planning in the Netherlands for professionals and citizens alike. The purpose of this research was to explore whether this goal had been achieved. The research showed that although the website has about 1,000 hits per day it is barely used for planning purposes, but rather for general consultations on spatial plans. The offline available GIS files, however, are frequently used in research and policy-making. The results of this research confirm the previously recognized problems associated with the implementation of online public participation GIS in planning practice.

"The New Map of the Netherlands" (DNK) shows plans made by municipalities, water boards, provinces and national government departments. It presents them in such a way that they can be easily compared. As a project it started in 1997, following the initiative of three Dutch associations for planning-professionals: BNSP, NVTL and NIROV. The goal of the project was to collect and draw all spatial plans made for the Netherlands on the same digital map. For that purpose, sponsors were found and a project group was established with the same name as the product. Since 2001 the Dutch Ministry of Spatial Planning, Social Housing and Environment became the main sponsor of the project, by financing the maintenance and updating of the database and the website. The project team of DNK is part of the Netherlands Institute for Spatial Planning and Housing (NIROV).

DNK has had two editions so far. The first one, published in 1997, involved about 2,700 plans for housing, offices, nature and infrastructure and it was presented as an Illustrator drawing. The second edition was published in 2002 and since then it was updated every half a year. It involves about 5,000 plans that are stored in the GIS database and are accompanied with information about the destination, plan maker, contents, time frame, phase in decision making, etc. Thus the actual basis of the second edition of the New Map is GIS, but the map is published and available in three modes — as a printed poster, as a GIS project and as an interactive map on the website of DNK (http://www.kaart.nieuwekaart.nl, Figure 1).

The goal of the interactive map is informing and involving citizens in spatial planning issues and thus supporting the democratization of spatial planning. It is unique in the Netherlands for its ability to give an up-to-date overview of spatial plans for the whole country, to be a blotting-pad for design on a regional scale, to make plans comparable because they are all drawn in

Fig. 1 — Interface of the interactive map of "The New Map of the Netherlands"

one scale and with the same legend and finally to give citizens an opportunity to open forums and discuss the issues among themselves. In that respect the interactive map can be categorized as a public participation GIS.

This paper presents the results of the research done on the use of the interactive map from the web site in planning practice. The results are based on an examination of the web statistics and two inquiries, one with accidental website visitors and another with actual website users.

This evaluation of the interactive map is only a part of the research done by The Netherlands Institute for Spatial Research (RPB) on the applicability of "The New Map of the Netherlands" (DNK) in the Dutch planning practice. The complete research project was established in order to estimate the value of DNK in all aspects of its use, which are: how the GIS database is used by planners in daily practice and for research, how the data are used by non-planning instances such as educational or commercial offices, and the extent to which the interactive map is used as PP GIS for the democratization of spatial planning. Due to the complexity of the research, this paper presents only the results of the examination of the use of the interactive map of DNK as PP GIS.

INTERACTIVE MAP AS PP GIS

By its nature the interactive map of DNK belongs to the category of public participation GIS. Such GIS systems have been developed in recent years with the main goal of enabling ordinary people and local communities to understand information that originate from professional GIS applications and in that way make themselves ready for public discussions. The reason behind this is the assumption that public participation increases with the level of access to information (W e i d e m a n n and F e m e r s, 1993).

The case of "The New Map of the Netherlands" partly confirms this opinion. The plans are presented in such a way that users can retrieve them by zip code, place, or the name of the plan. Users can also choose the way how to combine the plans, look for information about the maker of the plan, basic features of the plan and the link to the developer. In addition to this informative part of the website, there is a possibility for users to comment on the plans or start a discussion about some issue in the forum.

Thanks to these features of the interactive map, this PP GIS is technically able to accommodate a relatively high level of participation. Using the terminology of W e i d e m a n n and F e m e r s (1993) (Public Participation Ladder, Figure 2), it can be said that the interactive map gives participants the right to know and to object, provides information and possibilities to define their interests and to determine the agenda for discussion. In theory this should be enough to support participation of not only professionals, but also non-professionals, by direct communication and equal ability to access the information.
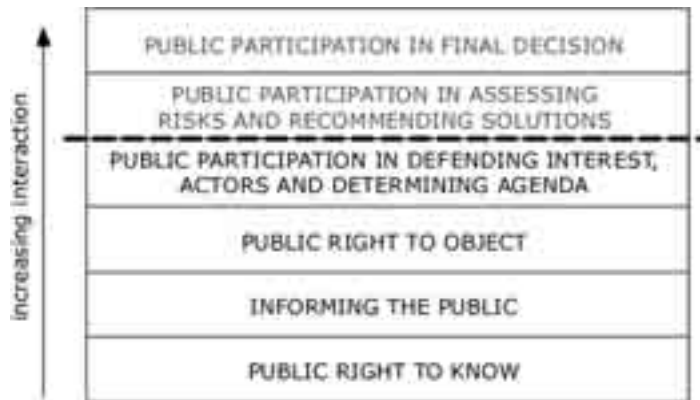
Fig. 2 — Position of DNK in the public participation ladder
(adopted from W e i d e m a n n and F e m e r s, 1993)

In order to get a better insight into the real usage of the interactive map, an inquiry was made by analyzing the website statistics, and conducting two interviews — one with the visitors to the website and another with persons who were considered to be potential users of the website. The inquiry took place in the period September—November 2004 and was made by two institutes — RPB and NIROV.

RESULTS OF THE INQUIRY

Results of the Analyses of Website Statistics

The project group of "The New Map of the Netherlands" used Webalizer as a web statistics counter. Counter statistics showed that there were about 1,100 visitors[2] per day in the period September — November 2004, which is quite a large number. The counter can be used to see how many unique visitors hit the website, whether they come directly or from some other website, whether they go further than the first page, and how long they stay.

The analyses showed that about one-quarter of the visitors came from another, often spatial planning related website. However, the majority of the visitors (74%) found the DNK website via a search engine, typing most often the words "map of the Netherlands", "map Netherlands", "map", "Netherlands map" etc. as search query. Only 0.98% of the visitors came to the website by typing the exact phrase 'de nieuwe kaart van nederland' in the search engine.

Results of the Interview with Visitors of the Interactive Map

As the counter is limited to some general information and it does not show the profile of the visitors, the project group of DNK decided to put a small questionnaire on the website which would pop up for every visitor leaving the website. This interview lasted from 23 September to 15 October and in those 23 days the website had 25,288 visitors. Despite the fact that the questionnaire contained only three questions and could be completed within three minutes, only 645 or 2.55% of the total number of visitors filled up the questionnaire.

Tab. 1 — Categories of users

Category	Number	Percentage
businesses	128	21
private	279	47
education and research	99	17
government	76	13
NGO	14	2
general	49	*

* The category "general" is not included in further analyses.

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[2] A visit occurs when a remote site makes a request for a *page* on your server for the first time. As long as the same site keeps making requests within a given timeout period, they will all be considered part of the same Visit. If the site makes a request to your server and the length of time since the last request is greater than the specified timeout period (*default is 30 minutes*), a new Visit is started and counted, and the sequence repeats. Since only *pages* will trigger a visit, remotes sites that link to graphic and other non-page URLs will not be counted in the visit totals, reducing the number of *false* visits. Due to the limitation of the HTTP protocol, log rotations and other factors, this number should not be considered as absolutely accurate, it should rather be considered a pretty close "guess". (http://www.mrunix.net/webalizer/webalizer_help.html)

The goal of the first question of this simple interview was to discover to which category of users each visitor belongs. The results showed that the majority of the visitors are private persons (Table 1).

The second question was meant to find out whether the visitors of the website also use plans in the interactive map. These visitors were named "users". All the visitors who came to the website only to find any kind of topographic map of the Netherlands were not counted as users. Furthermore, there was a portion of visitors who came to the website without any reason. Those were named 'surfers'. The division of visitors into users and non-users is shown in Table 2.

Tab. 2 — Users versus non-users

Category	Number	User	Uncertain maybe	Nont user	Surfers	Out of which potential users
private	267 (279)	49	3	139	76	59
businesses	82 (128)	31	29		22	
education and research	99 (99)	17	33	17	32	
government	71 (76)	41		17	13	
citizens	14 (14)	2	10	2		
total	533 (645)	140	75	175	143	

Table 2 shows that the majority of the visitors were private persons, but only a small portion of them were users of the information on plans that are available in the map. Approximately one-quarter of the visitors originated from the professional community, of which one-third belonged to governmental organizations.
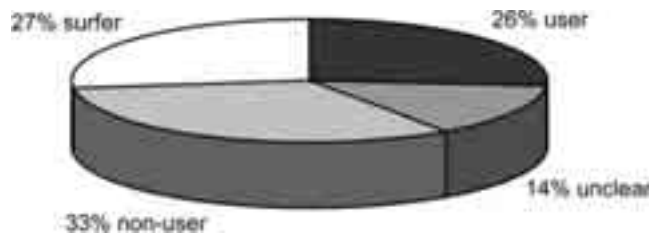
Fig. 3 — Users divided by type

Figure 3 shows that approximately one-quarter of the visitors actually used the interactive map to retrieve information on the plans.

The last of the three questions of the project group DNK query was about the satisfaction of the visitors with the result of their search. This question together with other remarks of the respondents led to the conclusion that the interactive map needs some improvements, especially concerning technical functioning, speed, update rate (of the presented information), and graphic presentation of maps.

The purpose of this evaluation conducted by The Netherlands Institute for Spatial Research was to learn the extent to which the interactive map is actually used in planning practice. Therefore, an interview was conducted among the professionals in spatial planning whose names were picked from the address book of the project group DNK. Those persons had either delivered data for the map development or were in some other way related to this project. In addition to those for whom it was certain that they were professionals in spatial planning, all subscribers to the newsletter of the DNK were also asked to participate in the interview. For this group it was not certain how many of them were professionals because anybody who visits the website can sign up for the newsletter and the project gGroup has no evidence about the origin of these persons.

The inquiry consisted of three levels — usability testing, empirical evaluation and assessment of the overall value of the interactive map (Figure 4). This approach is based on the method for evaluation of pPublic participation websites developed by T i š m a (2001) which was derived from A d e l m a n (1992) and A d e l m a n and R i e d e l (1997) approaches to the evaluation of knowledge-based systems and decision support systems.

Fig. 4 — Three levels of evaluation of the interactive map of DNK

According to this approach, technical properties of the system should be evaluated in the first instance. Secondly, it is important to know how users interact with the system and how they experience it. Thirdly, the way the system responds to the needs of an organization should be evaluated. The fourth level should consider whether this whole set — the system, the user and the organization — fits the needs of the professional community.

The first level testing — the technical properties of the web site — was not done because it had already been done by the project group DNK during the development of the website. Therefore the first set of questions started with the relationship between the user and the system. This was evaluated by employing usability testing. Usability addresses the question of how much the users like the system. The criteria used to measure it are a combination of criteria for the usability testing of websites and the usability of computer applications in general. Those are: *general ease of use, consistency, attractiveness, control, efficiency and learnability*[3] The questions in the inquiry were so formulated as to correspond with these criteria.

The second set of questions was related to the content of the interactive map and it was evaluated by using the empirical evaluation method. Empirical evaluation focuses on a user's performance with (versus without) the system. The reason for the empirical evaluation is that even when a system is technically well built and users like it, it still might not be used. The explanation for that may lie in the inadequacy of the content of the system's knowledge base or the inadequate process of its use. The criteria for empirical evaluation are: *process quality, product quality* and *overall confidence of the system.*

The third set of questions was about the general value of the interactive map for the planning of society.

There were in total 34 questions in the questionnaire and the respondents could answer on the scale from 1 (completely disagree) to 5 (completely agree) and add their own comment.

Results of the Poll with the Users of the Interactive Map

A total of 3,713 persons were contacted by e-mail and asked to fill in the questionnaire. Only 61 *of them* responded which *makes* 1.64%. Although the response was so low it was decided to continue with the analyses, to at least get some insight in the usefulness of the *i*nteractive map. As PP GIS systems are not very often evaluated it was considered that even the modest results of this research *could* be useful for other researchers and developers of *p*lanning *s*upport and PP GIS systems. The results presented in this paper should therefore be considered as indicative and not as definitive.

The respondents were analyzed according to age, sex, experience in use of the Internet, profession, frequency and reasons for visiting the interactive map site.

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[3] General ease of use means that the system is easy to use and understand. *Consistency* implies that the system is consequent in the layout of the screens and in presentation of information; that the same commands produce the same actions throughout the system and that all parts of the system are clearly labeled. *Attractiveness* tackles the degree to which users like the site, whether they find it pleasant to use. *Control* shows the degree to which users feel 'in charge', whether the site allows them to navigate through it with ease. *Efficiency* is the degree to which users feel that the site has the information they are looking for, that it works at a reasonable speed and is adapted to their browser.

Learnability is the degree to which users feel they can get to use the site if they come into it for the first time, and the degree to which they feel they can learn to use other facilities ot access other information once they have started using it.

All age categories were equally represented except the one between 50 and 70 years of age, which included a slightly larger number of respondents than the other categories. The largest part of the population were males (77%), mostly experienced Internet users. Most of the respondents originated from government and business (Table 3).

Tab. 3 — Respondents per origin

Category of respondent	%
Government	37
Businesses	26
Education	5
Research	2
NGO	4
Private	18
Other	5
Empty	3
Total	100

Most of the respondents visit the website once a month. The reasons for the visit are to look for plans, check what is planned in their or neighboring municipalities, to be up to date with spatial developments, to control whether the data that they delivered to the project group are properly presented and updated. Some of the respondents come because of their general interest in spatial planning, and some use the data about plans for their own purposes such as to predict the number of potential new pupils in schools or to estimate the potential market for some activities or products.

The majority of the answers which concerned usability testing were neutral but more on the positive than on the negative side with regard to criteria of the general ease of use, consistency, attractiveness, control, and efficiency. With regard to the criterion of learnability, the majority of respondents thought that it was not easy to use the system without some background knowledge about spatial planning and GIS.

The majority of respondents were satisfied with the process of how the map with the plans was coming into existence. They felt positively about the way the plans were categorized, what information about the plans were presented and they thought that the terminology used was familiar to them.

Talking about the product — the map with the plans on it — the respondents were not so satisfied with the visual presentation of the map and they had some doubts about whether the information were up-to-date or not. In general, the users thought that their search gave them a better insight into the spatial developments planned then they would have without using the system.

Respondents were neutral about the general reliability of the interactive map, but as with most other questions, there were more respondents on the positive than on the negative side of the scale. Whatever else this might mean, it also indicated that the respondents were not really acquainted with the possibilities of the interactive map and therefore were not able to give a precise answer.

The third part of the questionnaire contained questions about the value of the interactive map for spatial planning in the Netherlands because one of the main goals of the makers of the interactive map is to support the democratization of spatial planning. There were only four questions at this level of evaluation and the answers were somewhat extreme when compared with the other two levels of the evaluation.

In reply to the question as to whether the interactive map helps in understanding the complexity of the planned spatial transformations of the Netherlands, a significant number of answers was below the average. The next question was whether DNK is a good instrument for the democratization of knowledge about the spatial planning. Here 53% of the respondents were positive, 54% of respondents thought that the interactive map increases the transparency of spatial planning and 45% thought that DNK could help improve coordination between different layers of planning authorities in the Netherlands.

It is interesting to note that although only 45% of the respondents thought that they would come back to this website, still the great majority (about 70%) would like this project to continue and improve.

CONCLUSIONS: THEORY AND REALITY ABOUT THE INTERACTIVE MAP AS PP GIS

The goal of this research was to evaluate the interactive map of "The New Map of the Netherlands" as a public participation GIS. Since the first public presentation of "The New Map of the Netherlands" in 1997, this project received much attention in media and the professional community. In 2002, the interactive map was presented to the general public and launched with no restrictions. The expectations were that it would be broadly used in the planning practice. This research showed that although the website with the interactive map has about 1,000 hits per day, a small portion of visitors use the map for its primary purpose.

Two inquiries were made among the visitors and the users of the website, but both of them had very low response rates. A low response can lead us to two possible conclusions: (1) people do not want to waste time participating in on-line polls, and (2) a low response also means low use of the interactive map as PP GIS.

An explanation for both situations might be that there is an unfortunate combination of technical problems with the interactive map such as user friendliness, accuracy and actuality of data, and its novelty so that it needs time to become admitted into the current system of spatial planning. Although the interactive map is easy to understand by experienced GIS and Internet users, for non-professionals it requires time and effort to learn how to use it and to understand the real gain of using it. The investment in learning time is still much higher than the gains provided by the system, therefore, rational ignorance is quite high in this case. The impact that users of the interactive map can have on planning processes is low and it is never direct. Participants could even-

tually initiate some changes very indirectly, by giving comments on presented information or starting discussions about some issues with other visitors of the website.

Our analyses showed that "The Interactive Map of the Netherlands" is technically and contextually not a perfect PP GIS, although it has many useful features in itself. It is not institutionalized and therefore not embedded in the current social and political context, thus its potentials are not being fully exploited. However, as public participation GISs are expected to continue and increase, "The New Map of The Netherlands" has a potential to reach a level where it will provide adequate support to participants so that they can take an active part in actual planning processes.

This research confirmed the previously recognized problems with public participation GIS — technically they are still too complicated for non-professional users and they have a marginal position compared with traditional ways of planning development and approval. The question arises as to whether the democratization of public participation through electronic networks is a utopian idea, wishful thinking in a society which is neither technically nor institutionally ready to accept this novel approach. This issue should be given more attention in further research on PP GIS.

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ЕВАЛУАЦИЈА ГЕОГРАФСКОГ ИНФОРМАЦИОНОГ СИСТЕМА ЗА ЈАВНО УЧЕШЋЕ ГРАЂАНА У ОДЛУЧИВАЊУ О ПРОСТОРНИМ ПЛАНОВИМА

Александра Д. Тишма
Холандски институт за истраживање простора,
Виљема Витзенплајна 6, 2596 БК Хаг, Холандија

Резиме

Географски инфорамциони системи за јавно учешће у просторном планирању (PP GIS) су веома популарни инструменти у научним круговима. У пракси просторног планирања они се међутим веома мало примењују. Сматра се да главни разлози за овакву ситуацију леже у још увек компликованој структури и садржају ових инструмената. Са друге стране у устаљеној дневној пракси одлучивања о просторним плановима постоји отпор према прихватању нових инструмената.

PP GIS често нису оцењивани; у ствари, оцењивање ових система је једна од најзанемаренијих активности у циклусу њиховог развоја. Овде приказано истраживање даје неке нове информације о корисности PP GIS система у планерској пракси. Током истраживања евалуиран је PP GIS који се зове "Нова Карта Холандије". "Нова Карта Холандије" је GIS пројекат који сакупља све могуће просторне планове на територији државе, провинција и општина у једну базу података. Путем Интернета грађани имају приступ одређеном делу ове базе података. Систем је финансирало холандско Министарство за просторно планирање, социјално становање и животну околину, а развијен је у последњих пет година.

Евалуација је показала да се PP GIS "Нова Карта Холандије" не користи у оној мери у којој би се могло очекивати с обзиром да му је приликом првог публиковања поклоњена пажња у многим медијима.

Веома мали број посетилаца web странице на којој се налазе подаци о просторним плановима био је спреман да одговори на анкету овог истраживања. На основу одговора који су били сакупљени могу се извести само индикативни, а не и научни закључци, а то су: да је систем још увек превише компликован да би га користили обични грађани али и да грађани немају довољно мотива да троше своје време на учење система, јер осим што могу да се обавесте о плановима, њихова евентуална реакција на поједине планове нема директног утицаја на доношење одлука.

D a r i n k a   M .   B o g d a n o v i ć

Faculty of Agriculture
Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

THE ROLE OF PHOSPHORUS IN EUTROPHICATION

ABSTRACT: Eutrophication is an increase in the biological productivity of water basins resulting from the accumulation of biogenic elements under the influence of anthropogenic factors. Natural eutrophication is a slow and inevitable process. Artifical eutrophication is caused by human activity and it can be very rapid, especially in technologically developed countries. Eutrophication does not represent a mere change of water but a change in the metabolism of the entire ecosystem and hence a change of the ecosystem itself. In Serbia and Montenegro, all factors are present that promote eutrophication in both still (especially in lowland areas) and running waters. This paper deals with the results of eutrophication monitoring and prognostication for the DTD canal network and major rivers and lakes in Serbia, including a special review of the trophic status of lakes in the country's main lowland region, the Vojvodina Province. As phosphorus concentration is an important factor in the process of eutrophication of a body of water, the study has devoted particular attention to the control of this element.

KEY WORDS: eutrophication, organic matter, phosphorus, water trophic status

INTRODUCTION

The first study of eutrophication dates back to the early 19th century (D e C a n d o l l e, 1825) when a *Cyanophyceae* species *Oscillatoria rubescens* invaded the lake Murtensee in Switzerland. This bacterial species is still considered a reliable indicator of eutrophication.

Natural eutrophication is independent of human influence and its rate is variable. In regions rich in nutrients, shallow waters start as euthrophic. In regions poor in nutrients, there occur oligotrophic lakes. Natural eutrophication has a slow progress, it never reaches catastrophic proportions but it cannot be stopped.

Accelerated eutrophication caused by human action is called artificial eutrophication. The process may be rapid and it occurs primarily in countries boasting of high technological advances. The process may be interrupted only by discontinuing the supply of nutrients (E d m o n d s o n, 1969). If the release of household waste waters into water ecosystems continues, the saprobity of water keeps increasing. Some microorganisms take part in water self-purifica-

tion, mineralizing organic substances into inorganic compounds. This process increases the trophic level and water turns eutrophic (U h l m a n n, 1967). A similar process takes place in installations for purification of communal and industrial waste waters. If, however, waste water rich in nutrients is released into lentic waters, it enters the cycling of nutrients within the ecosystem resulting in artificial eutrophication with all of its negative consequences.

EUTROPHICATION IN SERBIA AND MONTENEGRO

In Serbia and Montenegro (SCG) and especially in the SCG lowlands, all factors that encourage eutrophication of lentic waters are in evidence. The interest in eutrophication has intensified in recent decades. This interest coincided with the OECD international project (1982) on the control, monitoring and forecast of eutrophication which has generated a series of reports that presented results gathered over a period of several years. Attention was focused on the control of phosphorus since a number of studies have indicated phosphorus as a key factor of eutrophication.

In all studies of eutrophication of lentic waters conducted so far in SCG, attention has been given to phosphorus and its various forms since it is considered the most important element in biological production, particularly in the processes of artificial eutrophication. Numerous literature data refer to phosphorus as a limiting factor in the biological production of organic matter (S c h i n - d l e r and L e a n, 1973; S c h i n d l e r and F e e, 1974).

Based on the Water Act (Official Gazette of the Republic of Serbia nos. 46/91, 53/93, 67/93 and 48/94) and the Regulations on the Monitoring of Water Quality brought by the Government of the Republic of Serbia, Hydrometeorological Bureau of the Republic of Serbia systematically monitors quantitative and qualitative characteristics of the surface and ground waters on the territory of Serbia. Tables 1 and 2 present the results of saprobiological analyses and phosphorus concentrations, two important water quality parameters, in the major water courses and reservoirs in Serbia.

Danube. The saprobiological analysis of water quality of the Danube River has indicated the presence of a moderate organic load. Bioindicators of β-mesosaprobic zone were dominant, accompanied by indicators of α-mesosaprobic and polysaprobic zones. The groups *Bacillariophyta* and *Chlorophyta* were the most frequent organisms. The saprobity index placed the water quality into class II.

Tisza. A moderate organic load. Predominance of indicators of β- and α-mesosaprobic zones, chiefly belonging to the groups of brown and green algae. The saprobity index was within the limits of b-mesosaprobic zone, i.e., quality class II.

Sava. A moderate organic load. Predominance of indicators of β-mesosaprobic zone. The saprobity index within the limits of quality class II.

Velika Morava. Predominance of indicators of β-mesosaprobic zone from the groups of brown and green algae. The saprobity index in quality class II.

Drina. A moderate organic load. Predominance of indicators of β-mesosaprobic zone. Water quality in class II.

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Canal DTD. The saprobiological analysis has indicated a moderate organic load. Predominance of indicators of β-mesosaprobic zone from the groups *Bacillariophyta*, *Euglenophyta* and *Chlorophyta*. The saprobity index within the limits of quality class II.

Tab. 1 — Concentration of phosphorus in major rivers and DTD canal network in Serbia and Montenegro in the year 2000 (mg/l^{-1})* (collected by Bogdanović)

Water resources	No. of measurements	PO^{3-}_4 from-to	P_{tot}
Dunav	15	0.00—0.088 X̄ 0.043	0.00—0.588 X̄ 0.116
Nišava	4	0.00—0.163 X̄ 0.068	0.016—0.459 X̄ 0.173
Velika Morava	6	0.033—2.085 X̄ 0.372	0.100—3.00 X̄ 0.518
Ibar	3	0.00—0.538 X̄ 0.160	0.093—0.581 X̄ 0.233
Tisa	5	0.007—0.121 X̄ 0.058	0.052—0.480 X̄ 0.194
Tamiš	3	0.010—0.157 X̄ 0.062	0.071—0.211 X̄ 0.152
Sava	4	0.00—0.139 X̄ 0.051	0.010—0.285 X̄ 0.090
Drina	4	0.00—0.048 X̄ 0.019	0.014—0.080 X̄ 0.045
DTD	8	0.003—1.630 X̄ 0.192	2.241—0.031 X̄ 0.370

* Hydrometeorological Bureau of the Republic of Serbia. [Hydrological Yearbook] No. 3, 2000.

Tab. 2 — Concentration of phosphorus in reservoirs and lakes in Serbia and Montenegro in the year 2000 (mg/l^{-1})* (collected by Bogdanović)

Reservoir	P tot	PO_4^{3-}
Gruža	0.083—0.049 X̄ 0.066	0.055—0.038 X̄ 0.046
Bovan	0.084—0.027 X̄ 0.055	0.044—0.025 X̄ 0.034
Ćelije	0.139—0.042 X̄ 0.090	0.042—0.037 X̄ 0.039
Grlište	0.065—0.043 X̄ 0.054	0.028—0.020 X̄ 0.024
Vrutci	0.054—0.049 X̄ 0.051	0.021—0.010 X̄ 0.015
Zlatibor	0.071—0.043 X̄ 0.057	0.027—0.018 X̄ 0.022

	Lake			
	Palić	Ludoš	Bačka Topola	Bela Crkva
P tot	0.259	0.300	0.118	0.048
PO_4^{3-}	0.020	0.023	0.028	0.008

* Hydrometeorological Bureau of the Republic of Serbia. Hydrological Yearbook No. 3, 2000.

Serbia and Montenegro is situated between 42° and 46° northern geographic latitude. Its area is 102,173 km². It is located in the zone of moderate climate, with a small part in the northernmost periphery of the subtropical climate. Serbia and Montenegro is predominantly mountainous, with the highest summit at 2656 m on the Prokletije Mountains and the lowest point at 28 m at the confluence of the rivers Danube and Timok.

The average annual rainfall in the mountainous part of the country ranges between 800 and 1200 mm. The annual rainfall is below 800 mm in the largest part of Serbia, while the northern parts of the country have the rainfall bellow 600 mm.

The population of Serbia and Montenegro is 10,394,026, with the population density of 101.7 inhabitants/km². According to the 1953 census, 72.9% of the population lived in the rural areas. In the 1991 census, their number was 28.4%.

In the year 2000, the total arable land in Serbia and Montenegro was 44,450,000 ha and the number of agricultural workers was 1,738,078 or 17.1%. In 2002, the number of private farms was 1,175,706 and the number of agricultural enterprises and cooperatives 1,550.

The total area of the Republic of Serbia is 88,361 km² and it can be roughly divided into three geographic units. The Vojvodina Province is a lowland, the central Serbia is a medley of plains, hills and mountains, while the Kosovo and Metohija Province is comprised of hills, mountains and valleys. Of the total arable land in the Republic, 85.1% are privately owned and 14.9% are state owned.

According to elevation, lowlands (up to 200 m above sea level) take 37% of the Republic of Serbia, hills (200—500 m) and low mountains (500—1000 m) each take 26% and high mountains (> 1000 m) take about 11%. Regarding inclination, there is a relatively high percentage (42.6%) of steep or very steep slopes (above 30°) covered with shallow soils prone to erosion and unsuitable for cultivation. Level sites (sloping less than 5°) and mild slopes (5—10°) take one third of the territory and the remaining 24% are slopes between 10 and 30°, which can be used for agricultural purposes after applying certain measures (H a d ž i ć et al., 2002). In the year 2000, eroded soils covered an area of 3,486 km² while the area recovered from erosion was 1,544 km². In the same year, the area protected from flooding was 1,376,000 ha of arable land, and the flooded area was 60,100 ha in Yugoslavia and 46,090 ha in Serbia. Drainage practices had to be applied at 2,073,000 ha, with the total canal network of 27,205 km. The portion of agricultural land in the total territory of the Republic of Serbia is 65%. The main categories of agricultural land according to use are presented in Table 3.

Tab. 3 — Agricultural land of the Republic of Serbia according to mode of utilization, in ha*

	Fields and gardens	Orchards	Vine-yards	Meadows	Arable land	Pastures	Fish ponds and marshes	Agricul-tural land
Vojvodina Province	1579643	16496	12196	32326	1640661	119176	30140	1789977
Central Serbia	1799399	228719	63265	547954	2639337	710358	5537	3355232
Kosovo and Metohija Province	291046	11816	9422	88353	400640	182352	83	583075
Republic of Serbia Total	3670091	257031	84883	668633	4680638	1011884	35760	5728282

* 2004 Statistical Yearbook of Serbia and Montenegro.

In the year 2000, the total consumption of mineral fertilizers was 304,000 t, or 108,000 t of active substance. The average amounts of mineral fertilizers and active substance per hectare were 68.4 and 24.3 kg/ha^{-1}, respectively. The number of domestic animals per hectare of arable land was 0.35.

Mineral fertilization in Serbia and Montenegro fell below normal in the early 1990's and this practice persisted to date. For the sake of comparison, 1,450,000 t of mineral fertilizers or 556,000 t of active substance were applied in 1985 to a comparable acreage. These amounts were five times as large as the currently used amounts. This is why the period 1980/90 is considered a period of intensive use of mineral fertilizers and chemicals in general. In the wheat-growing regions, fertilizers were applied on the basis of a system of soil fertility control, at the ratio 1:0.52:0.45 (N:P_2O_5:K_2O). During the period of UN sanctions, fertilizers application plummeted (P and K could not be imported) and the ratio became quite unfavorable, 1:0.2:0.2. A study of B o g d a - n o v i ć et al. (1993) gave global estimates of soil contamination with heavy metals and the contents of biogenous elements for 2,600,000 ha of arable land in Serbia (Table 4). A comparison of the 1993 results with the 2000 results from the soil fertility control system showed considerable reductions in the contents of potassium and especially phosphorus, which were obviously due to the systematic omission of P and K fertilization in the period 1990/2002.

Tab. 4 — Percentages of soil samples per classes of available phosphorus and potassium in Serbia in 1993 and 1996 (B o g d a n o v i ć et al., 1993; J a k o v l j e v i ć and S t e v a n o v i ć 1996)

Region	Area (ha)	No. of samples	P_2O_5	K_2O	P_2O_5	K_2O	P_2O_5	K_2O
			very poor to poor		optimum content		high to very high	
Central Serbia	1,000,000	1,000	59.0	8.0	17.0	34.0	25.0	37.0
Vojvodina Province	1,600,000	1,600	26.1	9.8	67.4	83.2	6.5	5.0

The high contents of phosphorus (67% in the optimum class, 6.5% in the high class) in the soils of the Vojvodina Province were the consequence of in-

tensive P fertilization before 1990 (Table 4). As previously mentioned, the average NPK ratio in Serbia and Montenegro in the 1970's was 1:2.5:1.2, to fall to 1:0.2:0.2 in the year 2000. This trend caused reductions in the yields of agricultural crops, which was undesirable, but it also lowered a risk of pollution (eutrophication of waters by nitrates and phosphorus). On account of this short supply of fertilizers and pesticides, the largest portion of the arable land in Serbia and Montenegro is free of the risk of contamination with heavy metals and other harmful substances, which makes it suitable for the production of safe food. According to Č u v a r d i ć et al. (1999), the balances of the three most important nutrients in the soils of Serbia and Montenegro are negative: –23 kg N/ha; –17 kg P_2O_5/ha and –16 kg K_2O/ha. The same authors claim that 100 kg N/ha, 50 kg P_2O_5/ha and 80 kg K_2O/ha, one domestic animal per hectare and 30% of legumes in the crop rotations are needed to make the balance positive. According to B o g d a n o v i ć et al. (1993; 1997), based on long-term experiments (25 years) on intensive exploitation of the chernozem soil, a positive balance of nutritive elements is achieved by applying 100 kg N/ha, 50 kg P_2O_5/ha and 40 kg K_2O/ha. The nitrogen fertilization was based on the N min method, the phosphorus and potassium fertilization on the balance method. Available phosphorus and potassium were analyzed at 4-year intervals. Results of soil analyses were used for planning PK fertilization of the crops grown.

The most frequent agricultural sources of contamination of surface waters, water from the first aquifer and the deeper ground waters are mineral fertilizers, liquid manure, communal and industrial waste waters. The Ministry for Science, Technology and Development of the Republic of Serbia finances national projects which cover only a part of studies of importance for environmental protection. The provided funds are insufficient for conducting country-wide experiments on a long-term basis. Therefore, this paper deals with the results of a 10-year study "Eutrophication of lentic waters of the Vojvodina Province" conducted by Đ u k i ć et al. (1991). Additionally, it covers the results of eutrophication monitoring in two mountain and two lowland reservoirs and two lowland lakes, conducted in the period 1989/90 (Table 5). The most important parameter from the point of eutrophication is the way a reservoir is supplied with water.

Borkovac Reservoir, built in 1975, is located on the southern slopes of the Fruška Gora loess plateau. Its size at normal water level is 41.4 ha, its drainage area is 23.8 km^2. The reservoir was constructed primarily for improvement and intensification of agricultural production. It is surrounded with orchards and vineyards, forest and tilled land. As the reservoir also serves as a flood wave regulator, it permanently receives certain amounts of nutrients.

In a 10-year study (1980/90) of Đ u k i ć et al. (1991), the total amount of dissolved salts was 410 g/m^3. Trophicity parameters are given in Table 5.

Based on the 10-year trophicity parameters in Table 5, Borkovac Reservoir may be classified as mesotrophic, noting that oligo-mesotrophic elements tend to occur from time to time. Based on the presence of heterotrophic bacteria, according to the water classification of K o h l (1975), the water of Bor-

Tab. 5 — Trophicity parameters of some lakes and reservoirs in the Vojvodina Province (1980/90) (Đukić et al., 1991)

Trophicity parameter	Minimum and maximum value				
	Reservoir Borkovac	Reservoir Sot	Reservoir Zobnatica	Ludoš Lake	II accumulating lake Palić
Orthophosphates	0.015—0.080 g/m³	0.011—0.079 g/m³	0.004—0.118 g/m³	0.088—0.846 g/m³	0.535—2.136 g/m³
Total P	0.098—0.946 g/m³	0.090—0.656 g/m³	0.101—1.880 g/m³	0.238—4.350 g/m³	1.119—3.68 g/m³
Mineral N	0.280—1.160 g/m³	0.132—0.835 g/m³	0.22—2.86 g/m³	0.250—5.637 g/m³	2.854—12.88 g/m³
Total N	1.128—4.223 g/m³	0.746—3.806 g/m³	1.377—6.355 g/m³	—	6.32—27.59 g/m³
Chlorophylla	3.33—77.32 mg/m³	2.41—27.97 mg/m³	9.5—148.7 mg/m³	17.94—255.8 mg/m³	3.52—173.02 mg/m³
Primary organic production	112—416 mgCm⁻²day⁻¹ from oligo- to mesotrophic	17—205 mgCm⁻²day⁻¹ oligotrophic — oligo-mesotrophic	187—427 mgCm⁻²day⁻¹ mesotrophic	485—1050 mgCm⁻²day⁻¹ meso-polytrophic	294—848 mgCm⁻²day⁻¹ from meso- to meso-eutrophic

kovac Reservoir gradually passed from class II to class II—III. Water quality continually deteriorated over the observation period.

Based on the measurement of enzymatic (phosphatase) water activity, Borkovac Reservoir has passed from category II A (satisfactorily pure) to category III (contaminated and very contaminated) over recent years.

Sot Reservoir was constructed in the immediate vicinity of the Fruška Gora Mountain and it was put in operation in 1981. The geological substrate of the reservoir consists of crystal schists predominantly covered with loess. The size of the reservoir is 22 ha, with the drainage area of 13.6 km². The reservoir, surrounded with orchards and forested land, was constructed for irrigation and to prevent intensive local erosion. The average amount of dissolved salts in the period 1980/90 was 313 g/m³. Trophicity parameters are given in Table 5. The level of trophicity varied in the period 1980/90 as indicated by the parameters given in Table 5, placing the reservoir into the category of mesotrophic waters with oligotrophic characteristics occurring periodically. The results of enzymatic (phosphatase) water activity indicated a trend of gradual deterioration of water quality. In the course of the observation period, the Sot Reservoir passed from category II and III (moderately contaminated) to category III A (contaminated).

Zobnatica Reservoir was constructed in the region of Bačka in 1978. It is supplied from the Krivaja Brook which receives certain amounts of waste water from the village of Bajmok, and it may be potentially contaminated with nutrients coming from the pig farm in Đurđin. The size of the reservoir is 250 ha. Arable land reaches to the very edges of the reservoir and this is how nutrients contaminate the reservoir. The total amounts of salt ranged from 487 to 700g/m³. They were found to vary in dependence of climatic conditions, intensity of mineral fertilization in the drainage area and the amounts and composition of the waste waters reaching the reservoir. The water in the reservoir is of the Na-HCO$_3$ type. This type is characteristic for and widely spread in the Pannonian Plain. Trophicity parameters are given in Table 5.

Considerable differences were found when comparing the 10-year values of total phosphorus for the Zobnatica Reservoir on one side and the Fruška Gora Reservoirs (Borkovac and Sot) on the other. The former reservoir had much larger amounts of phosphorus, the annual increments indicating the presence of a process of accelerated eutrophication. The Zobnatica Reservoir is classified among eutrophic waters, with periodic mesotrophic and polytrophic elements. The results of enzymatic (phosphatase) water activity indicated a trend of gradual deterioration of water quality in the course of the study period. The average annual values of the index of phosphatase activity of water showed that the water gradually passed from class IIA to classes IIIB, III and IV. Studying the effect of agricultural production on water contamination in reservoirs of the Vojvodina Province in the period 1980/89, V a j a g i ć and B e l i ć (1990) noted for Zobnatica Reservoir a degradation of water quality even in the case of well-drained soils.

The Ludoš Lake. Its size is 330 ha. It is provided with water through the canal Ludoš-Palić, which receives purified water from Sector II of the Palić Lake and from the Kereš Brook. It is located in an urban area 12 km away from the town of Subotica. It is surrounded by private farms which use its water for irrigation. The total amounts of dissolved salts ranged from 992 to 1,257 g/m³. Over the 10-year study period of Đ u k i ć et al. (1991), the chemical type of water changed from NaCl to $NaHCO_3$ and then to Na_2SO_4. Trophicity parameters are given in Table 5. The average annual values of the index of phosphatase activity of water showed that the water had a very low quality. R a t a j a c (1988) found that the eutrophication of that lake was intensive. Studying nutritive elements and trophic level of the lake in the period 1982/87, S e l e š i (1988) found that the primary organic production kept increasing, classifying it as eupolytrophic with the plankton-type eutrophication and recommending a beginning of reclamation.

The Palić Lake is one of the oldest lakes in Yugoslavia (originating from 1781). The main source of the phosphorus load of the Palić Lake is definitely the purified waste water from the town of Subotica.

In the period 1977—1998, the following results of P were obtained for the first accumulating pond:

— Mean annual amount of purified water, 11,424,145 m³;

— Mean P content in purified water, 9.01 g/m³/year;

— Total P reaching the first accumulating pond, 103 t/year (S e l e š i, 2000).

This enormous amount of P is incorporated into living organisms in the first and second accumulating pond. P uptake by the living organisms is very intensive since the P content in the first accumulating pond (9.01 g/m³) is reduced to 2.9 g/m³ in the lake intended for public recreation and tourism. The average results of the 19-year study are (calculated per 10 million m³ of water, the remaining water considered as having evaporated):

— Total amount of water, 10 000 000 m⁻³/year

— Total amount of P, 19-year average, 2.9 g/m³

— Total P reaching the tourist part lake, 29 tons/year

Taking in consideration that the amount of P in the dry residue of algae is 1%, it ensues that the tourist part of the Palić Lake can annually produce 2,900 t of algal dry residues from the amount of P coming to the lake (thence the thick and intensively green water of the lake). The installed filters are capable of decomposing the organic matter in the urban waste water, but salts go directly into the lake. In the presence of O_2, the bacterial filters mineralize organic matter into inorganic salts and they also are deposited in the lake. In that way the lake takes double pressure: from nutritive salts originally contained in industrial waste water and from nutritive salts formed as the end product of organic matter degradation in the purification technology. This enormous amount of nutritive salts (primarily phosphorus and mineral N) is used by algae which multiply in masses, giving the dark green color to the lake water and reducing its transparency. P and N not only represent a load for the lake water but also

they are taken up by living organisms, thus entering the cycle of nutrients, remaining in the lake for a protracted period and causing undesirable changes. To successfully protect lake waters (of the Palić and other lakes), it is necessary: to reduce or discontinue the application of mineral fertilizers in the immediate vicinity of the lake, to prevent soil erosion, to establish a protective belt around the lake, with grassland, shrubbery and forest, to reduce animal production to a minimum in the immediate vicinity of the lake, etc.

CONCLUSION

Studies conducted so far on the occurrence of eutrophication in Serbia have led to the following conclusions:

The most common sources of agricultural pollution of surface waters, first aquifer waters and groundwaters are mineral fertilizer and liquid manure application and community and industrial wasterwaters.

The amount of salts (primarily phosphorus and mineral nitrogen) in water depends on climatic conditions, mineral fertilizer use in the catchment area and the amount and composition of wastewaters that flow into the accumulation.

Monitoring of trophic status parameters in accumulations and lakes has revealed that the quality of water has dropped severely with a tendency towards further eutrophication (Table 5). This calls for protective measures to be taken, otherwise the waters will become unusable for their intended purposes.

In order to slow down the process of eutrophication in the lakes and accumulations, mineral fertilizer use should be reduced or even completely discontinued, soil erosion around the lakes should be prevented, buffer zones should be established that consist of lawns, shrubbery or even woods, and all animal husbandry activity in the surrounding area should be reduced to a minimum.

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УЛОГА ФОСФОРА У ЕУТРОФИКАЦИЈИ

Даринка М. Богдановић

Пољопривредни факултет, Трг Доситеја Обрадовића 8, 21000 Нови Сад

Резиме

Еутрофикација је повећање биолошке продуктивности водених басена, а настаје као последица сакупљања биогених елемената под дејством антропогених фактора. Природна еутрофикација је веома спора и не може се зауставити. Вештачка еутрофикација је изазвана човековим деловањем и може бити веома брза

посебно у земљама са развијеном технологијом. Појава еутрофикације није нека проста промена воде, него промена метаболизма читавог екосистема, а резултат тога је промена и самог екосистема. У нашој земљи су, такође, присутни сви они атрибути који поспешују еутрофикацију стајаћих вода, посебно у равничарским реонима, али и текућих вода. Отуда се у раду износе резултати мониторинга и прогнозе еутрофикације за канал ДТД и веће реке и језера, са посебним освртом на параметре трофичности језера у равничарском делу Србије — Војводини. Како је концентрација фосфора у водама значајан фактор еутрофикације, отуда је и посебна пажња посвећена контроли овог елемента.

На бази параметара трофичности воде у акумулацијама и језерима у Војводини (таб. 5), квалитет воде у праћеном периоду јако се погоршао, са тенденцијом даље еутрофикације, те се морају предузети мере заштите јер ће у противном вода бити неупотребљива за намене за које је пројектована.

S v e t l a n a   I.   C a c i g[1]
M a r i a - R a l u c a   I.   S z a b o[2]
A l f a   X e n i a   D.   L u p e a[1]

[1] Facultatea de Chimie Industrială şi Ingineria Mediului
Universitatea "Politehnica" din Timişoara
P-ta Victoriei Nr. 2, Timişoara 1900, Romania
e-mail: svetlana_cacig@yahoo.com

[2] Facultatea de Inginerie Alimentară, Turism şi Protecţia Mediului
Universitatea "Aurel Vlaicu" Arad
Str. Elena Drăgoi nr. 2, Arad, 2900, Romania

SPECTROPHOTOMETRIC METHOD FOR THE STUDY OF THE ANTIOXIDANT ACTIVITY APPLIED ON *ZIZIPHUS JUJUBA* AND *HYDRANGEA PANICULATA* AQUEOUS EXTRACTS

ABSTRACT: Antioxidant activity of water extracts from *Ziziphus jujuba* fruits and *Hydrangea paniculata* leaves, maintained in a refrigerator, was determined by the potassium permanganate method. The results presented in this paper indicate that the fruit extracts from *Ziziphus jujuba* had higher antioxidant activity than *Hydrangea paniculata* leaf extracts and that both fermentation processes had a strong effect on antioxidant activity.

KEY WORDS: antioxidant activity, plant extract, spectrophotometric method

INTRODUCTION

It is a current trend in different domains, especially food production and medicine, to search for natural products that can replace the synthetic ones that can *Ziziphus jujuba* fruits and *Hydrangea paniculata* leaves are known to be rich in flavonoids (P r i d h a m, 1965; C l e m e n t et al., 2004). and this is why we have studied their antioxidant properties versus ascorbic acid, a synthetic and water-soluble antioxidant.

MATERIAL AND METHODS

Material: Potassium permanganate and sulfuric acid were purchased from "Reactivul", Bucureşti, the ascorbic acid from Merck and the YPD agar medium from Teknova.

Apparatus: Spectrophotometer SPEKOL 10 (Carl Zeiss, Jena) fitted with 30 ml quartz vats and magnetic stirrer and coupled with an acquisition plate (Ai-chrom 727 computer interface Aion Chromatography, Inc., USA) and a Pentium II computer for the antioxidant activity determination.

Plant material: Fruits of *Ziziphus jujuba* (E1) and leaves of *Hydrangea paniculata* (E2) were collected in October 2004 and were dried at 20°C in the dark place. After that, 4 g of the dried sample was finely chopped into small parts and then extracted with 60 ml water for 70 h at 10°C, followed by filtration. The final volume of the extract was 40 ml.

Antioxidant properties screening by the potassium permanganate assay: The method is based on the redox reactions between the antioxidant sample and the potassium permanganate in sulfuric acid media, leading to sample discoloration. Previously done experiments (I a n c u l o v et al., 2001) determined only the time until no color could be observed. We have improved this method by the spectrophotometric pursuit of the process, which allows a more accurate calculus. We have already published a series of results obtained by this method on various spices (S z a b o et al., 2005; C a c i g et al., 2005).

Variable amounts of samples (v ml), depending on the intensity of the antioxidant activity, were introduced in a 30 ml quartz vat containing an oxidative mixture of: 1.5 ml potassium permanganate 0.01M; 3.5 ml sulfuric acid 2M and (20-v) ml distilled water. That moment was considered the zero time. The spectrophotometer signal (mV) was then registered at 535 nm until constant value. Subsequent variation ariations of the potassium permanganate concentration were afterwards determined based on a previously prepared calibration curve. The extracts were kept in the a refrigerator (10°C) and the analyses were done within 40 days from the beginning of their storage.

RESULTS AND DISCUSSION

The spectrophotometer signal appearing on the monitor represents mV values. A calibration curve was determined by preparing a series of six solutions with different concentrations of potassium permanganate and registration of the electronic signal (mV) for each of them. Pairs of data were graphically presented and linearly fitted. The obtained graphic and the corresponding equation are presented in Figure 1.

Based on this, the potassium permanganate concentration (x) can be determined from the signal value (y) using the formula:

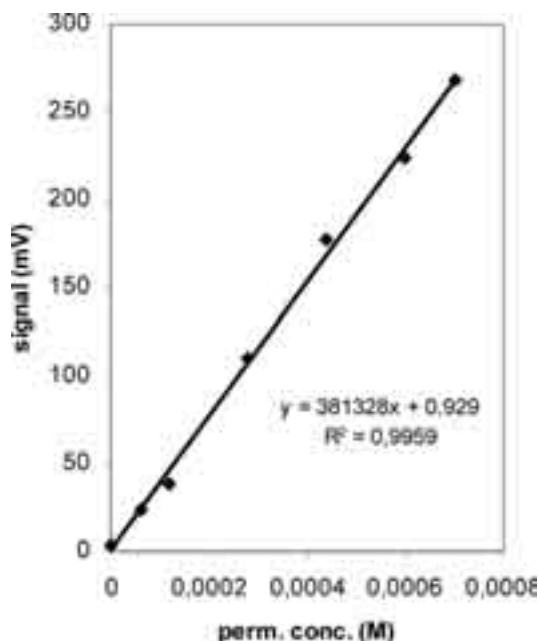$$x = \frac{y - 0{,}929}{381328} \tag{1}$$

Fig. 1 — Calibration curve for the potassium
permanganate in the oxidative mixture

The corresponding curves for the extracts kept in the refrigerator, are shown in Figure 2a, b after 1, 2, 4, 8, 12, 18, 24, 32 and 40 days from their preparation.

Most curves show a decrease in the potassium permanganate concentration, as expected. Still, in some cases, the rapid decrease is followed by a false increase, due to the formation of MnO_2 particles, which afterwards precipitate.

It is probable that during the first step, corresponding to the rapid decrease of the concentration, reacts substances with higher antioxidant activity whitch are able to reduce Mn (VII) to Mn (II). After that, reacts substances with lower antioxidant activity which reduces Mn (VII) up only to Mn (IV), — precipiting MnO_2 leading to the precipitate formation. During the first phase of this phenomenon, the formed MnO_2 particles thus formed induces the light diffusion and temporarily increase total fake absorption. Later on, a part of the MnO_2 precipitates, and another part is probably dissolved by the sulfuric acid in the medium and this is why the absorption decreases again.
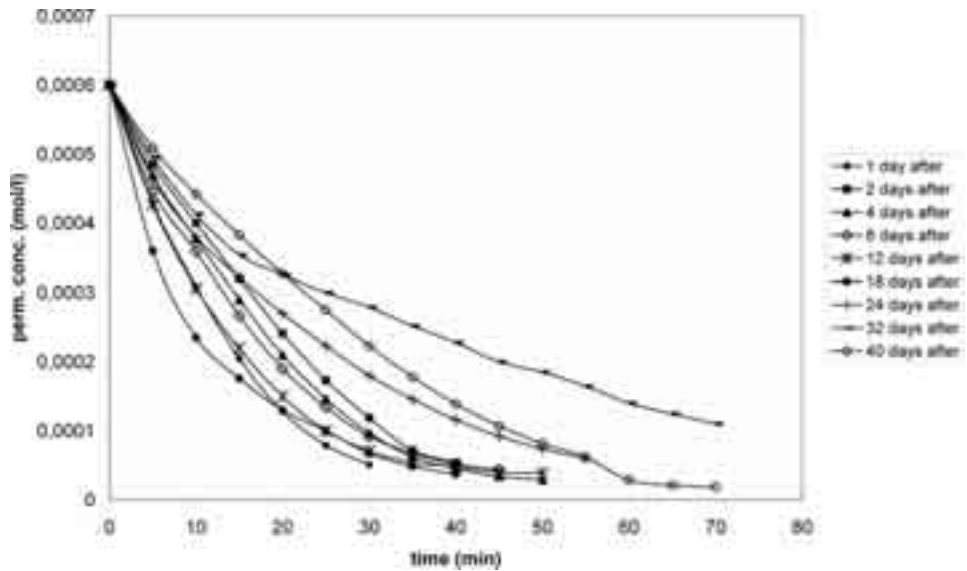
Fig. 2a — Variation of the potassium permanganate concentration after adding 0.2 ml
of *Ziziphus jujuba* fruits (E1), 1, 2, 4, 8, 12, 18, 24, 32 and 40 days
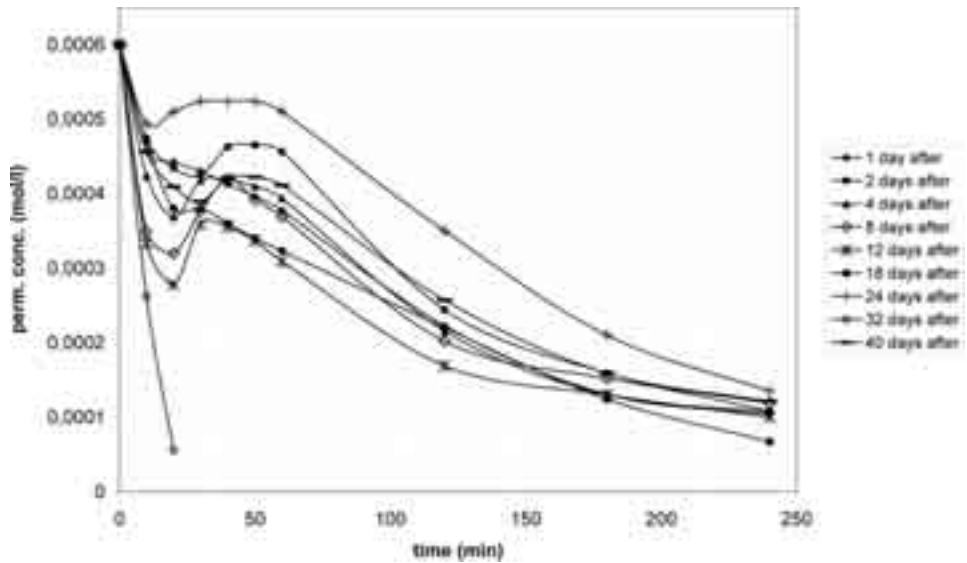from their sample preparation

Fig. 2b — Variation of the potassium permanganate concentration after adding 0.2 ml
of *Hydrangea paniculata* leaves (E2), 1, 2, 4, 8, 12, 18, 24, 32 and 40 days
from their sample preparation

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In order to quantitatively compare the antioxidant activities, we proposed the following formula:

$$A_{50} = \frac{t_{standard}}{t_{plant\ sample}} \cdot \frac{C_{standard}}{m_{plant}} \cdot \frac{V_{standard}}{V_{plant\ sample}} \cdot V_{extract} \quad (2)$$

where:

A_{50} — antioxidant activity expressed, reflected in the time until the sample induces a decrease of the oxidizing agent [potassium permanganate] concentration up to one half, compared against a standard [ascorbic acid] (mmol equivalent standard / g plant)

$t_{plant\ sample}$ — the time until the sample induces a decrease of the permanganate concentration up to one half (min)

$t_{standard}$ — the time until the standard (ascorbic acid) induces a decrease of the permanganate concentration up to one half (min) [0.65 minutes as seen in Figure 3]

$C_{standard}$ — standard (ascorbic acid) concentration (mmol/ml) [0.01 mmol/ml]

m_{plant} — weight (g) of the plant sample submitted to extraction [4g]

$V_{plant\ sample}$ — volume of the plant extract submitted to the analysis [0.2 ml]

$V_{standard}$ — volume of the standard submitted to the analysis [1 ml]

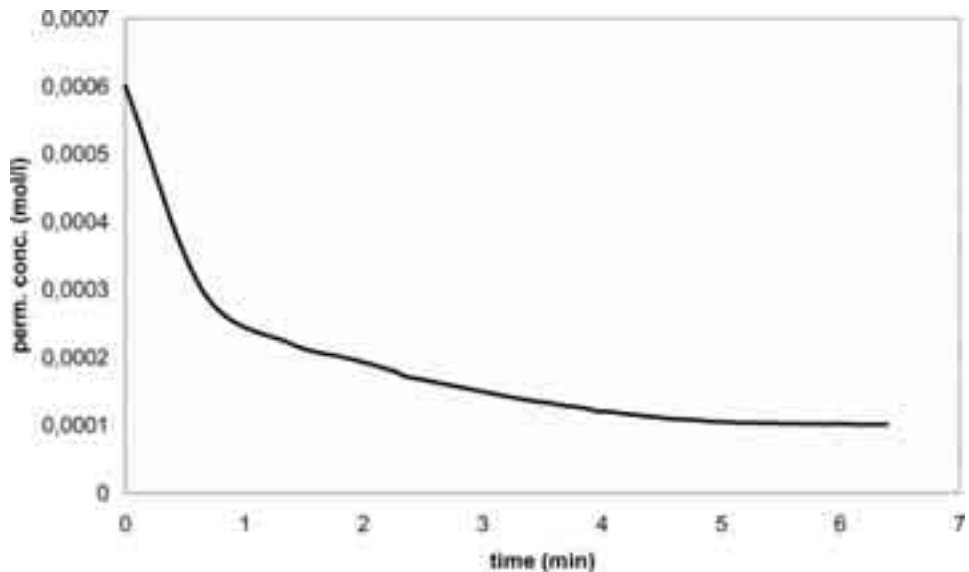$V_{extract}$ — volume (ml) of the obtained extract [40 ml]

Fig. 3 — Variation of the potassium permanganate concentration after adding 1 ml of ascorbic acid 0.01 mol/l (mmol/ml)

The variations in the A_{50} values are given in Table 1.

Tab. 1 — A_{50} values for the studied extracts

Day	A_{50} (mmol equivalent ascorbic acid/g plant)	
	Ziziphus jujuba	*Hydrangea paniculata*
1	0.1935	0.0228
2	0.1231	0.0200
4	0.1401	0.0223
8	0.1563	0.0245
12	0.1935	0.0317
18	0.2902	0.0278
24	0.1195	0.0145
32	0.0813	0.2257
40	0.0903	0.0200

The differences in the A_{50} values were related to the observed fermentation processes. It previously noticed (C a c i g, 2005) that the samples maintained at the room temperature, produce different patterns (C a c i g, 2005), compared to those maintained in the refrigerator. Temperature has influenced, not only on the reaction kinetics, but also on the microorganisms present. Our assumption that there were at least two types of microorganisms was proved correct after sowing on YPD (yeast peptone dextrose) agar medium (Figure 4).

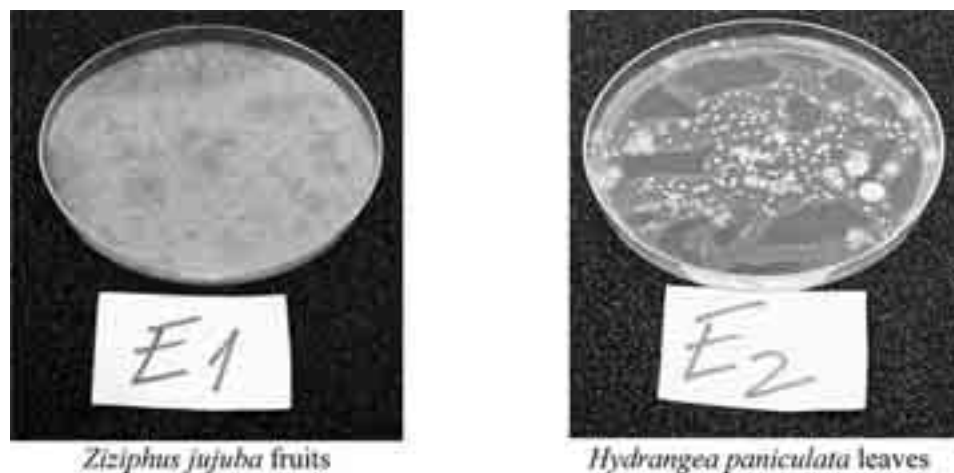*Ziziphus jujuba* fruits   *Hydrangea paniculata* leaves

Fig. 4 — Results of the YPD agar medium sowings, 4 days later

CONCLUSION

The spectrophotometric method we propose allows the quantitative appreciation of the antioxidant activity for the tested aqueous plant extracts.

With a single exception, *Ziziphus* fruit extracts seemed to have higher antioxidant activity than *Hydrangea* leaf extracts.

Fermentative processes seem to improve the antioxidant activity of the aqueous extracts, but further studies are necessary in order to determine the influence of ethanol, which is probably produced during the fermentation processes, on the obtained results.

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СПЕКТРОФОТОМЕТРИЈСКА МЕТОДА ЗА ОДРЕЂИВАЊЕ
АНТИОКСИДАТИВНЕ АКТИВНОСТИ ВОДЕНИХ ЕКСТРАКТА
ZIZIPHUS JUJUBA И *HYDRANGEA PANICULATA*

Светлана И. Каћиг[1], Марија-Ралука И. Сабо[2], Алфа Ксенија, Д. Лупеа[1]
[1] Факултет за индустријску хемију и амбијентално инжењерство, Универзитет „Политехника" Темишвар, Викторијин трг 2, Темишвар 1900, Румунија
e-mail: svetlana_cacig@yahoo.com
[2] Факултет за прехрамбену индустрију, туризам и заштиту животне средине, Универзитет „Аурел Влајку" Арад, улица Елене Драгој 2, Арад 2900, Румунија

Резиме

У раду је изучавана антиоксидативна активност воденог екстракта *Ziziphus jujuba* (плод) и *Hydrangea paniculata* (лист).

Активност је одређена на бази реакције антиоксидативних узорака са калијум перманганатом у киселој средини.

Резултати испитивања показују да екстракти добијени из плода *Ziziphus jujuba* имају већу антиоксидативну активност него екстракти из листа *Hydrangea paniculata*.

N a t a š a   P.   N i k o l i ć[1], *B o r i v o j   Đ.   K r s t i ć*[1]
S l o b o d a n k a   P.   P a j e v i ć[1], *S a š a   S.   O r l o v i ć*[2]

[1] Department of Biology and Ecology, Faculty of Natural Sciences
Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia
[2] Faculty of Agriculture, Institute of Lowland Forestry and Environment
Antona Čehova 13, P.O. Box 117, 21000 Novi Sad, Serbia

VARIABILITY OF LEAF CHARACTERISTICS
IN DIFFERENT PEDUNCULATE OAK GENOTYPES
(*QUERCUS ROBUR* L.)

ABSTRACT: The objective of this study was to determine genotype influences on pedunculate oak's leaf traits: leaf area, specific leaf area (leaf area per unit of leaf mass), concentration of photosynthetic pigments, rates of photosynthesis and respiration, and nutrient concentrations (nitrogen, phosphorus, potassium, calcium, and sodium). Leaf samples were taken from seventeen *Q. robur* genotypes originating from clonal seed orchard Banov Brod (Srem, Vojvodina, Serbia). Leaf area of the studied genotypes ranged from 248.4 to 628.8 cm^2, SLA from 109.4 to 160.7 cm^2 dry matter^{-1}, rates of photosynthesis and respiration from 6.98 to 20.32 and from 6.73 to 14.65 μmol O$_2$ m^{-2} s^{-1}, respectively. The leaves of genotype 35 contained the highest concentration of photosynthetic pigments, while the lowest were recorded in genotype 29. The following pattern of nutrient concentrations was obtained for the studied genotypes: N > Ca > K > P > Na. Genotype variability of P, K, Ca, and Na concentrations was more pronounced when compared with nitrogen. Estimated quantitative differences are the consequence of interaction of certain genotype and common environmental conditions for all trees. These results will provide information on intraspecific variation of the studied leaf characteristics.

KEY WORDS: *Quercus robur*, SLA, leaf area, photosynthesis, photosynthetic pigments, respiration, N, P, K, Ca, Na

INTRODUCTION

Leaves are the main photosynthetic organ in trees. Being highly sensitive, they are continuously subjected to environmental conditions as well as to phenological cycles and growth rhythms (B u s s o t i et al., 2000). Same environmental conditions could cause similar answers of leaf characteristics in trees. C a s t r o - D i e z et al. (1997) showed that some *Quercus* species could exhibit different responses (mirrored in leaf morphology and leaf chemical composition). Among other morphological parameters, specific leaf area (leaf area

per unit of leaf mass) is often used for characterizing leaf structure. Leaves will have a lower SLA if they are denser (greater mass per volume) or thicker (R e i c h et al., 1998). Because of its correlation with leaf gass exchange and leaf nitrogen, SLA has been extensively studied (W r i g h t et al., 2001).

Previous studies of certain leaf characteristics have dealt with differences between individual tree species, while intraspecific variations remained less known. Speaking of the latter, the results for properties of an entire genetically heterogeneous population may differ from those relating to identifiable subsamples within a population. Contrary to their expectations, B r u s c h i et al. (2003) found that among-tree variation of morpho-anatomical traits of *Q. petraea* leaves was lower than within-tree variation.

Element concentrations in organs of tree species are characterized by wide variability, depending on soil properties, climate factors, season and community structure (M u l e v et al., 1990). The element accumulation in plant leaves is a dynamic process that is coupled with other metabolic processes occurring in the plant (G i e r t y c h et al., 1997). Beside environmental conditions mentioned above, it is influenced also by leaf and plant age and leaf type. Foliar nutrient contents are often used to describe plant nutritional status and requirements (M a r s c h n e r, 1986).

Our hypothesis was that morpho-physiological characteristics of leaves differ among genotypes within the same stand and that these differences are under genetic control. Hence, objectives were to compare the variability of selected traits in *Quercus robur* genotypes, as well as to select genotypes possessing desirable characteristics for possible use in seed production, breeding and reforestation projects. These objectives were obtained by comparison of leaf morphology (leaf area, SLA), and physiology (concentration of photosynthetic pigments, rates of photosynthesis and dark respiration, leaf concentrations of N, P, K, Ca, and Na) in pedunculate oak genotypes grown under the same field conditions.

MATERIALS AND METHODS

Study area

The plant material for this investigation was obtained from the clonal seed orchard Banov Brod, situated along the left bank of the river Sava (44°55' N, 19°23' E), 81 m above sea level. The soil in the orchard belongs to eutric cambisol type. Its basic chemical characteristics were analyzed at Laboratory for Agroecology of Institute of Field and Vegetable Crops (Report no. 08-96/837) and data are shown in Table 1. The plantation consists of 85 *Q. robur* genotypes, and was established by grafting. To assess leaf morphological and physiological traits seventeen genotypes were chosen: 4, 5, 6, 16, 18, 20, 21, 22, 25, 28, 29, 30, 33, 35, 38, 40, and 85.

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Tab. 1 — Soil chemical properties

CaCO$_3$ (%)	Humus (%)	Nitrogen (%)	AL-P$_2$O$_5$ mg/100 g	AL-K$_2$O mg/100 g	pH in KCl	in H$_2$O
0.98	3.48	0.233	6.1	24.1	6.3	6.5

Plant material

Leaf samples were taken from 20-year-old trees grown under identical environmental conditions. In order to reduce the within-tree variability, one branch was harvested from the middle of the crown of each genotype chosen. Branches were immediately transported to the laboratory in closed plastic bags. Only undamaged, completely expanded (mature) current-year leaves (the same shoot and flash) were used for analysis.

Leaf morphological characteristics

The mean leaf area of individual genotypes (cm^2) was determined by portable leaf area meter LI — 3000 (Li-Cor, USA), using twenty mature leaves. These leaves were dried in paper bags at 105°C to constant weight, and their mass was measured to estimate a value for specific leaf area (cm^2 dry matter^{-1}).

Leaf physiological characteristics

The concentration of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and total carotenoids) was estimated spectrophotometrically after extraction in absolute acetone and expressed as mg g^{-1} dry matter (W e t t s t e i n, 1957).

The rates of net photosynthesis and the dark respiration were determined polarographically, using a oxygen electrode (W a l k e r, 1989). The rate of photosynthesis was evaluated by the quantity of released oxygen (μmol O$_2$ m^{-2} s^{-1}), and the dark respiration by the quantity of absorbed oxygen (μmol O$_2$ m^{-2} s^{-1}).

Following drying at 105°C, the content of leaf dry matter was measured and expressed as percentage of fresh leaf mass. Dried leaf samples were milled and used for chemical analysis. Total N concentration was estimated by standard micro-Kjeldahl method (N e l s o n and S o m m e r s, 1973). After dry ashing at 450°C and treatment with HCl, samples were analyzed for P, K, Ca, and Na concentrations. Phosphorus concentration was determined spectrophotometrically by ammonium-vanadate-molybdate method (G e r i c k e and K u r - m i e s, 1952). Potassium and sodium concentrations were estimated using flame photometry (M a r j a n o v i ć and K r s t i ć, 1998), and calcium by atomic absorption spectrophotometry (VARIAN SPECTRA A-10). Nutrient concentrations were expressed in mg%, i.e., mg per 100 g dry matter.

Statistical analyses

The obtained results were processed statistically by the analysis of variance using the program MSTATC. The comparison of genotypes was done by Duncan's test. The mean values of the studied parameters were ranked and marked with letters. Values with the same letter did not differ significantly at $p < 0.05$.

RESULTS

Leaf morphology

Table 2 summarizes the values of leaf area and SLA. Leaf area varied from 248.4 to 602.1 cm². Genotypes 18 and 20 could be separated from others by the lowest (248.4 and 263.6 cm², respectively), while genotypes 6 (601.9 cm²), 29 (628.8 cm²) and 38 (602.1 cm²) by the highest values. Compared with the leaf area, SLA showed almost two fold lower variability among the studied genotypes. It ranged from 109.4 (genotype 40) to 160.7 cm² g⁻¹ (genotype 35).

Tab. 2 — Morphological parameters of leaves in different *Q. robur* genotypes. CV — coefficient of variation (%); LA — leaf area (cm²); SLA — leaf area per mass (cm² dry matter⁻¹)

Genotype	LA	SLA
4	490.9 bc	144.0 abc
5	574.3 ab	117.6 ef
6	601.9 a	148.5 ab
16	321.6 efg	129.5 bcde
18	248.4 g	136.6 bcde
20	263.6 g	141.2 abcd
21	485.1 c	120.2 ef
22	391.1 de	145.3 ab
25	386.2 def	121.3 def
28	360.1 def	132.1 bcde
29	628.8 a	124.4 cdef
30	291.7 fg	123.9 def
33	369.6 def	148.8 ab
35	434.1 cd	160.7 a
38	602.1 a	144.8 ab
40	323.7 efg	109.4 f
85	317.1 efg	134.8 bcde
Average	417.1	134.3
CV	12.5	7.80

Leaf physiological characteristics

The data related to rates of photosynthesis and respiration are given in Table 3. The rate of net photosynthetic oxygen evolution depended on geno-

type. The highest photosynthetic activity was estimated in genotype 35 (20.32 μmol O_2 m^{-2} s^{-1}), the lowest in genotype 40 (6.98 μmol O_2 m^{-2} s^{-1}). The greatest differences in dark respiration rate was obtained between genotypes 38 (6.73 μmol O_2 m^{-2} s^{-1}) and 21 (14.65 μmol O_2 m^{-2} s^{-1}).

Tab. 3 — Rate of photosynthesis (μmol O_2 m^{-2} s^{-1}) under light saturation and the rate of dark respiration ($-\mu$mol O_2 m^{-2} s^{-1}) in *Q. robur* genotypes. CV — coefficient of variation (%).

Genotype	Net photosynthesis	Dark respiration
4	11.12 ef	9.17 def
5	11.87 def	9.67 cdef
6	11.06 ef	9.67 cdef
16	11.84 def	11.37 bcd
18	9.45 fgh	11.62 bc
20	13.15 cde	10.15 cdef
21	17.40 b	14.65 a
22	11.26 ef	10.65 bcde
25	11.87 def	10.40 bcdef
28	12.90 de	11.84 bc
29	14.48 cd	10.12 cdef
30	14.96 bc	8.67 efg
33	13.07 cde	9.90 cdef
35	20.32 a	8.95 ef
38	8.09 gh	6.73 g
40	6.98 h	12.62 b
85	10.62 efg	8.23 fg
Average	12.38	10.26
CV	12.35	11.89

The studied genotypes also differed considering the concentration of photosynthetic pigments and the dry matter content (Table 4). On average, leaves contained 4.53 mg g^{-1} of chlorophyll *a*, 1.31 mg g^{-1} of chlorophyll *b*, and 1.16 mg g^{-1} of carotenoids. Leaf dry matter content in individual genotypes varied between 31.4 (genotype 35) and 43.0% (genotype 40).

Tab. 4 — Concentration of chloroplast pigments (mg g^{-1} dry matter) and dry matter (%) in different *Q. robur* genotypes. CV — coefficient of variation (%).

Genotype	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids	Dry matter
4	5.19 bcd	1.64 b	6.83 bcd	1.26 bcd	42.7 a
5	5.35 bc	1.36 cd	6.71 cde	1.43 ab	33.8 fg
6	5.70 ab	1.93 a	7.63 ab	1.41 abc	36.2 de
16	4.43 efg	1.42 bcd	5.85 efg	1.19 def	38.0 cde
18	3.69 hij	0.86 e	4.55 i	1.06 efg	38.5 bcd
20	3.32 jk	1.02 e	4.33 i	0.84 h	37.2 cde
21	3.88 ghij	1.05 e	4.93 hi	1.03 fg	37.6 cde
22	4.31 fgh	1.26 d	5.57 fgh	1.09 defg	35.9 ef
25	4.55 def	1.43 cd	5.98 def	1.22 de	40.4 b
28	4.97 cde	1.43 bcd	6.40 cdef	1.24 cde	38.7 bc
29	2.78 k	0.66 f	3.45 j	0.78 h	36.4 cde
30	4.08 fghi	0.96 e	5.06 ghi	1.02 fg	36.0 def

33	5.04 bcde	1.55 bc	6.59 cde	1.19 def	33.5 gh
35	6.19 a	1.88 a	8.07 a	1.49 a	31.4 h
38	5.35 bc	1.56 bc	6.91 bc	1.28 bcd	37.2 cde
40	3.49 ij	0.85 ef	4.34 i	0.92 gh	43.0 a
85	4.71 cdef	1.37 cd	6.07 cdef	1.22 de	37.7 cde
Average	4.53	1.31	5.84	1.16	37.3
CV	7.91	8.78	7.88	8.76	3.44

Table 5. summarizes leaf concentrations of macronutrients (N, P, K, Ca) and Na in different *Q. robur* genotypes. Compared with the other nutrients analyzed, nitrogen concentrations were highest and they showed lowest genotype differences. The highest nitrogen accumulation was recorded in genotypes 25, 35, and 38, the lowest in genotype 30 (3188 mg%). Phosphorus concentrations varied between 100 and 164 mg%. Highest concentrations were obtained in genotypes 25, 35, and 85, the lowest in genotype 20. On average, the concentration of this nutrient for all genotypes amounted to 136 mg%. According to potassium concentrations, genotypes can be classified into several groups. Genotypes 35 and 85 showed the highest accumulation of this macronutrient in leaves (858 and 850 mg%, respectively), whereas genotype 40 the lowest (408 mg%). The average calcium concentration was 2603 mg%. Its minimum and maximum values were 1,756 and 3,279 mg%, respectively. The greatest difference was found between genotypes 21 and 29. Leaf sodium concentrations were low, ranging from 25 to 56 mg%. Highest concentrations were recorded in genotypes 25 and 35 (56 and 53 mg%, respectively). Genotype 29 was characterized by the lowest calcium concentration, as well as the lowest sodium accumulation.

According to total ash content, the studied genotypes could be classified into four groups (Table 5). Generally, mineral matter content varied between 4.13 and 7.21%. Genotypes 20, 29, and 40 were distinguished from the others by lowest, and genotypes 4, 21, 25, 30, and 38 by highest ash contents.

Tab. 5 — Leaf concentration of mineral elements (mg%) and total ash content (%) in different *Q. robur* genotypes. CV — coefficient of variation (%).

Genotype	N	P	K	Ca	Na	Total ash
4	4108 bc	123 ef	617 de	2924 abc	32 cdefg	6.72 a
5	3848 e	141 cd	633 de	2838 abc	38 bcd	6.07 b
6	3739 f	135 de	550 ef	3087 ab	30 efg	6.10 b
16	3695 fg	119 fg	717 bcd	2147 defg	43 b	5.35 c
18	3869 e	120 fg	792 ab	2030 efg	34 cdef	5.87 b
20	3623 g	100 h	467 fg	1951 fg	38 bcd	4.52 d
21	4145 abc	153 abc	650 cde	3297 a	27 fg	6.79 a
22	3695 fg	146 bcd	533 ef	2453 cdef	30 efg	5.33 c
25	4210 a	164 a	592 de	2921 abc	56 a	6.80 a
28	4174 ab	146 bcd	767 abc	2845 abc	43 b	6.04 b
29	3688 fg	107 gh	458 fg	1756 g	25 g	4.62 d
30	3188 h	117 fg	463 fg	2870 abc	35 cde	6.77 a
33	4000 d	155 abc	608 de	2504 cde	34 cdef	6.22 b
35	4203 a	164 a	858 a	2603 bcd	53 a	6.00 b
38	4203 a	160 ab	708 bcd	3119 ab	39 bc	7.21 a

40	3710 fg	107 gh	408 g	2451 cdef	38 bcd	4.13 d
85	4079 cd	163 a	850 a	2452 cdef	43 b	5.19 c
Average	3893	136	628	2603	38	5.87
CV	1.25	5.86	10.73	10.82	11.10	5.11

DISCUSSION

Under similar growth conditions, the studied genotypes differed significantly in various leaf characteristics. However, intraspecific natural variability is known, and uniformity of growth conditions for all plants suggests that differences between genotypes were of genetic origin.

Genotypes 6, 29, and 38 could be separated by largest leaf areas, while genotypes 18 and 20 developed leaves with smallest areas. Genotype 38 developed the largest leaves. According to K a s t o r i (1998), morphological leaf features such as leaf area, leaf longevity, and SLA may be important in characterization of photosynthesis. Having almost thickest leaves and smallest leaf area among the studied plants, genotype 40 may be separated from the others by the lowest SLA. On the other hand, the highest SLA was estimated in genotype 35, which developed relatively thin leaves. Furthermore, the leaves of genotype 40 had the lowest photosynthetic activity, calculated on leaf area basis, while the highest oxygen evolution rate under light saturation was measured in genotype 35. Considering these results, it seems that leaf structure in genotype 35 enhances light harvesting resulting in the highest net photosynthetic activity. The question is: does a change in SLA alter the amount of light that can be intercepted per unit leaf mass? Results of E v a n s and P o o r - t e r (2001) suggested that SLA does not affect the whole leaf efficiency of light capture by the pigments. Therefore they concluded that in the absence of structures altering leaf reflectance (e. g., waxes and hairs), leaf absorbance is simply related to the chlorophyll content per unit leaf area. But, across a range of species, acclimation to a low-light environment is characterized by increased SLA, which was found to be far more important in maximizing carbon gain per unit leaf mass.

Interspecific divergence of photosynthesis in tree species has often attracted scientific interest because photosynthesis is a process strongly affected by ecological factors such as air temperature (H a m e r l y n k and K n a p p, 1996), CO_2 concentration (B e e r l i n g, 1996; K u b i s k e and P r e g i t z e r, 1996; A t k i n s o n et al., 1997; W a r d and S t r a i n, 1999), and soil O_2 concentration (P e z e s h k i et al., 1996). On the other hand, there was no data dealing with intraspecific, i. e., genotypic variability of this process in trees, including oak species. The cognition of biological sense of photosynthesis gives priority to genotypes characterized by high net photosynthetic rate. It is hard to reliably define genotypic specificity for photosynthetic activity since this physiological process is significantly affected by environmental factors and the ontogenetic stage of the photosynthetic organ and these factors manifest themselves in a wide time range (P a j e v i ć et al., 1999).

A comparison between data for maximum photosynthetic activity under light saturation in laboratory conditions and the concentration of photosynthetic pigments suggests a positive correlation ($r^2 = 0.12$; data not shown). Moreover, genotype 40, showing the lowest photosynthetic activity, was one of those having the lowest concentrations of chloroplast pigments, while the highest values of these parameters were obtained in genotype 35. This positive correlation was unexpected, because a lack of correlation between photosynthetic parameters and pigment concentration has been noticed previously in other plant species (E d w a r d s et al., 1993; P a j e v i ć et al., 1999).

Although photosynthesis has been the focus of much measurement and modeling, less is known about plant respiration, i.e., foliar respiration rates for many tree species are currently unknown (M i t c h e l l et al., 1999). The results of our study showed certain genotypic diversity in the rate of dark respiration in *Q. robur*. On the whole, the average dark respiration rate was –10.26 μmol O_2 m^{-2} s^{-1}. Genotype 21 could be singled out for the highest, genotype 38 for the lowest rate of dark respiration.

Chemical analysis showed that *Q. robur* leaves contained, on average, 3,893 mg% of nitrogen, 2,603 mg% of calcium, 628 mg% of potassium, 136 mg% of phosphorus, and 38 mg% of sodium. Compared with mean annual concentrations of bioelements in *Q. fraineto* and *Q. cerris* leaves (M u l e v et al., 1990), our results were higher for N, Ca, and Na, lower for K, while phosphorus concentration was between these two species. Similar patterns of nutrient concentration were reported for *Q. robur* and *Prunus avium* L. x *pseudocerasus* L i n d. (A t k i n s o n et al., 1997), and for Ca, K, and P in *Q. velutina* leaves (S p e c t o r, 1956). According to K o m l e n o v i ć and C e s t a r (1987), leaves of forty-year-old pedunculate oak trees contained less N and Ca and more P and K when compared with our data. Although the mineral nutrition is species specific (S a r i ć and L o u g h m a n, 1983), these differences could be the consequence of plant age, climatic and edaphic conditions.

Considering all leaf characteristics studied, leaf area showed the highest variability (12.5%). These results are consistent with findings of B u s s o t t i et al. (2000), who reported greater variability of leaf morphological parameters than leaf nutrient concentrations in three tree species. Somewhat lower variability was estimated for net photosynthetic rate (12.35%) and dark respiration rate (11.89%). Coefficient of variation of photosynthetic pigments and SLA was about 8%, while dry matter content revealed a low level of genotypic variability (3.44%).

The results presented above suggest that *Q. robur* genotypes did not exhibit large divergence regarding the studied leaf traits. These quantitative differences, illustrating intraspecific variability of the studied parameters, are the consequence of interaction of certain genotype and common environmental conditions for all trees. Within-species variability of leaf morphology and structure may improve plant performance, allowing species to maintain their fitness over a wide range of environmental conditions and resource availabilities (C a s t r o - D i e z et al., 1997). G a r n i e r et al. (1997) concluded that interspecific differences in structural and chemical characteristics of leaves are maintained under a wide range of growing conditions. Additionally, conside-

ring common environmental conditions for all of the studied plants, these results can provide information on the degree of genetic control of these parameters. These results could be used in selection of genotypes having desirable characteristics such as high photosynthetic activity. However, variations between genotypes should be considered with caution because of their strong connection with ecological factors; light conditions, before and among others, definitely influence leaf characteristics (e.g. leaf area, leaf mass, leaf thickness, SLA).

CONCLUSION

The results presented above suggest that the estimated intraspecific variations of the studied *Q. robur* leaf traits were slight but statistically significant. Considering all genotypes, leaf area, net photosynthetic and dark respiration rates showed the highest variability of all studied leaf characteristics. Genotype 35 could be distinguished for the highest concentrations of N, P, K, Na, photosynthetic pigments, as well as the rate of photosynthesis.

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ВАРИЈАБИЛНОСТ ОСОБИНА ЛИСТА КОД РАЗЛИЧИТИХ
ГЕНОТИПОВА ХРАСТА ЛУЖЊАКА (*QUERCUS ROBUR* L.)

Наташа П. Николић[1], Боривој Ђ. Крстић[1],
Слободанка П. Пајевић[1], Саша С. Орловић[2]
[1] Департман за биологију и екологију, ПМФ,
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Србија
[2] Институт за низијско шумарство и животну средину,
Антона Чехова 13, 21000 Нови Сад, Србија

Резиме

У раду је проучаван утицај генотипа на особине листа храста лужњака: лисну површину, специфичну лисну површину (површина листа по јединици масе листа), концентрацију фотосинтетичких пигмената, интензитет фотосинтезе и дисања, као и концентрацију нутријената (азота, фосфора, калијума, калцијума и натријума). Узорци листова су узети са седамнаест генотипова храста лужњака (*Quercus robur* L.), који потичу из вегетативне семенске плантаже Банов Брод (Срем, Војводина, Србија). Узимајући у обзир све генотипове, лисна површина се кретала од 248.4 до 628.8 cm^2, специфична лисна површина од 109.4 до 160.7 cm^2 сува материја$^{-1}$, интензитет фотосинтезе и дисања од 6.98 до 20.32, односно од 6.73 до 14.65 μmol O$_2$ m^{-2} s^{-1}. Највећа концентрација фотосинтетичких пигмената утврђена је код генотипа 35, а најмања код генотипа 29. Код свих генотипова утврђен је следећи однос концентрација нутријената: N > Ca > K > P > Na. Генотипска варијабилност концентрација P, K, Ca и Na била је већа него азота. Утврђене квантитативне разлике резултат су интеракције одређеног генотипа и истих услова средине. Добијени резултати пружиће информације о интраспецијској варијабилности проучаваних особина листа.

Dragana S. Latković
Ljubinko Đ. Starčević
Branko J. Marinković

Faculty of Agriculture, Novi Sad
Trg Dositeja Obradovića 8
21000 Novi Sad, Serbia

DYNAMICS OF DRY MATTER SYNTHESIS DURING CORN DEVELOPMENT

ABSTRACT: Effects of mineral nutrition and combinations of mineral and organic nutrients on the dynamics of vegetative mass development in corn have been examined in a long-term stationary trial. The dynamics was monitored per phenological stage and per plant part.

The obtained results showed that the dynamics of dry matter accumulation changed in dependence of development stage and nutrition variant. Smallest gains in dry matter in all stages under study were obtained in the control variant. Highest gains were obtained in the variants which combined mineral nutrition and manuring. The smallest gain of dry matter, 7.22% of the dry matter accumulated at full maturity, was registered at the seven-leaf stage.

KEY WORDS: corn, phenological stages, nutrition variants, dynamics of dry matter synthesis

INTRODUCTION

To ensure optimal conditions for corn nutrition, it is necessary to know the dynamics of dry matter accumulation and the dynamics of nutrition elements uptake during corn growth and development. High doses of NPK nutrients have a positive effect on total dry matter yield and grain dry matter. Dry matter accumulation in the corn hybrid NSSC 70 is very slow at the early stages of growth and development (S p a s o j e v i ć, 1972). The rate of dry matter accumulation starts to increase at the stage of 7—9 leaves and it continues to increase till the stage of full maturity.

In the period from emergence to silk stage (in dependence of climatic conditions of the year and fertilization variant) 68% of final yield are formed (M a r i n k o v i ć, 1986).

New hybrids have an increased rate of dry matter accumulation from pollination to physiological maturity, low translocation from the stalk and large green leaf area (T o l l e n a a r, 1991; R a j č a n and T o l l e n a a r, 1999).

Nitrogen fertilization increases dry matter weight and nitrogen concentration in grains and leaves (E l - H o u t, 1987; C o u s s o, 1983).

Dynamics of dry matter formation and nitrogen content and removal increase in proportion with increases in nitrogen fertilization, but more with low N doses and less with high N doses (S t a r č e v i ć et al., 1999).

S a r i ć and J o c i ć (1993) investigated the effect of NPK on main crops yield and concluded that the contributions of nitrogen, phosphorus and potassium to corn yield were 99%, 0% and 0.3%, respectively. Highest yields were achieved with highest amounts of nitrogen fertilizer. However, yield differences were not significant in relation to the yield achieved with the medium dose of nitrogen.

MATERIAL AND METHOD

Experiments have been conducted within the framework of a long-term stationary trial established in 1965 at Rimski Šančevi experiment field of Institute of Field and Vegetable Crops. The experiments included corn monoculture and a two-crop rotation of corn and winter barley. This study deals with two-year results from corn monoculture and two-crop rotation.

The trial had been established on a chernozem soil with good chemical, physical and biological properties.

The following fertilization variants were examined:

1. Control
2. NPK mineral nutrition
3. Mineral nutrition + stover
4. Mineral nutrition + manure (25 tha^{-1} every two years)
5. Mineral nutrition + manure in two-crop rotation

The two-crop rotation was manured each year, the monoculture at two-year intervals.

Starting from 1986, three nitrogen levels were applied in the above variants except the control:

— 60 kg N ha^{-1} in the fall
— 60 kg N ha^{-1} in the fall + 60 kg N ha^{-1} in the spring
— 60 kg N ha^{-1} in the fall + amount calculated by the N-min method in the spring.

The variants were grown as split plots in four replications.

The corn hybrid NSSC 640 was used in the experiment. It was machine-sown into the final row spacing 70x25 cm (57,142 plants/ha).

Samples of plant material were taken during the following phenological stages:

— Stage of 7 fully developed leaves
— Stage of silking

— Stage of wax maturity
— Stage of full development

The samples consisted of 12 plants (3 plants per replication). For dry matter determination, average samples were made, which were subsequently dried at 105°C. The samples were analyzed for percentage of dry matter, yield of dry matter and proportion of plant parts.

At the 7-leaf stage, we determined the percentages of dry matter in the whole plant and in the following plant parts: stalk, leaf, tassel and ear (grain, cob, husks, ear shank). Dry matter yield was expressed in kgha^{-1}.

RESULTS AND DISCUSSION

Accumulation of dry matter during particular stages of corn growth and development can be a reliable indicator of final grain yield.

In this study, the accumulation of dry matter in the aboveground plant parts was highly variable in individual stages of growth and development. Accumulation rate depended on stage of development and nutrition variant and it increased with the increase in mineral and organic nutrients. The dynamics of dry matter accumulation per nutrition variant and phonological stage is shown in Figure 1.

At the 7-leaf stage, dry matter (the average for the whole plant) was 1,127 kgha^{-1}, which was 7.22% of the total dry matter at full maturity. This was an indication that the intensity of dry matter accumulation at the early stages was low.

Similar low increments at the initial stages of growth and development were obtained by S p a s o j e v i ć (1972), B o g d a n o v i ć (1978), Ć i r o - v i ć (1985) and M a r i n k o v i ć (1986). Mineral nutrition significantly influenced the dynamics of dry matter formation. Significant differences between fertilization variants were obtained already at the stage of 7 leaves. The lowest dry matter was found in the control variant (566 kgha^{-1}) (Figure 1a), but mineral nutrition and the combination of mineral and organic nutrition increased the amount of dry matter. The highest dry matter was found in the variant of manuring the two-crop rotation + NPK (1,418 kgha^{-1}) (Figure 1e). Our results were similar to those obtained by J o c i ć (1978), but they were different from those of S p a s o j e v i ć (1972). The latter claimed that, at the initial stage of development, high NPK doses only slightly increased dry matter content, without statistically significant differences.

Intensive growth finishes with the silk stage (the stage of most intensive dry matter accumulation). In relation to the previously analyzed stage, the silk stage differed considerably in dry matter accumulation. Dry matter content was increased in all variants 8.45 times in relation to the previous stage. In the control variant, 72% of the final yield was formed. In the fertilized variants in monoculture, this value ranged from 61.0 to 65.6%. In the variant of manured two-crop rotation + NPK, 50.4% of dry matter was accumulated in the period from emergence to silking. These results were similar to those of M a r i n -
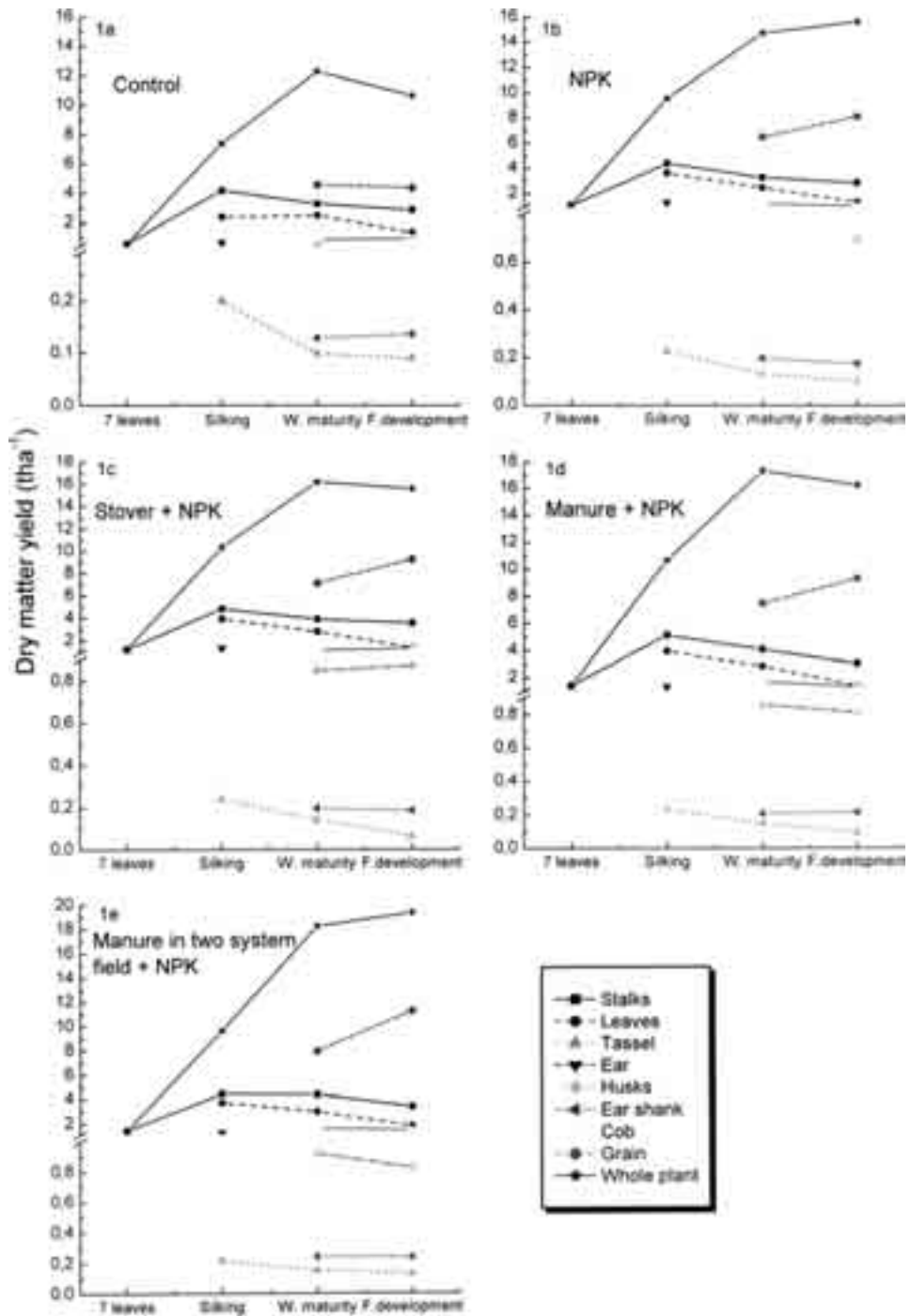
Fig. 1 — Dynamics of maize dry matter accumulation (tha^{-1})

k o v i ć (1986), who obtained 65% of final yield in the control variant and 60% in the fertilization variants. S p a s o j e v i ć (1972) obtained a smaller percentage of dry matter, about 50%. Ć u p i n a and J o c i ć (1972) determined that in this period 31—32% of final yield was formed. Our results do not agree with these results.

There were significant differences in dry matter content in individual plant parts. At the silk stage, stalks and leaves had maximum dry matter contents, and these results matched the results of other researchers (S p a s o j e - vić, 1972; J o c i ć, 1974; M a r i n k o v i ć, 1986).

A larger part of the total plant weight was accumulated in the vegetative plant parts (stalks and leaves) and a smaller part in the generative organs (tassel and ear). Smallest amounts of dry matter in all plant parts were found in the control variant. Highest dry matter contents in stalks and leaves were obtained in the variant with manure and stover (Figures 1c and 1a). Regarding the fertilized variants, ear dry matter was highest in the variant stover + NPK (1,343 kgha^{-1}), and smallest in the two-crop rotation (1,250 kgha^{-1}). The tassel had small dry matter, which was fairly uniform in the analyzed variants.

In the period from silking to full maturity, stalk and leaf mass kept decreasing. The reason for this was translocation of nutrients into the grain. The two-year average decrease in stalk weight was 31.5% in the control variant. In the fertilized monoculture, the decreases in stalk weight were 34% in the NPK variant, 27% in stover + NPK variant and 40.5% in manure + NPK variant. The decrease in the two-crop rotation was 24%.

From the silk stage to full maturity, leaf weight decreased by 42% in the control variant and 64% in the fertilized monoculture. In the two-crop rotation, leaf mass decreased by 52%. In relation to the results of S p a s o j e v i ć (1972) and M a r i n k o v i ć (1986), we obtained more significant decreases in the stalk and leaf weight.

In the ear, dry matter reduction was recorded from wax maturity to full maturity. Largest reductions were observed in the husks — 42% in the control variant, 19.7% in NPK variant, 5% in manure + NPK variant and 10% in the two-crop rotation. Our results are higher than those obtained by M a r i n k o - v i ć (1986).

Dry matter accumulation continued during the stage of wax maturity, but with smaller intensity. At the stage of wax maturity, stalk and leaf dry matter continued to decrease — the accumulated dry matter was translocated into generative plant parts, mostly in grains. Grain dry matter was smallest in the control variant (4,638 kgha^{-1}) (Figure 1a) and highest in the variant of manuring + NPK in the two-crop rotation (7,954 kgha^{-1}) (Figure 1e). Highest values of stalk weight were registered in the two-crop rotation and the variant of manuring + NPK in the monoculture. Leaf dry matter was highest in the two-crop rotation (2,978 kgha^{-1}). The differences between the control and the other fertilization variants were not significant. Dry matter in the cob was highest in the variant of manuring + NPK (1,684 kgha^{-1}) (Figure 1d) and lowest in the variant stover + NPK (1,156 kgha^{-1}) (Figure 1c).

Highest dry matter values in the husks and ear shank were found in the two-crop rotation and the control variant; the values in the other variants did not differ significantly.

Maximum dry matter yields at the stage of wax maturity were achieved in the control variant and the variant of manuring + NPK. In the other fertilization variants, maximum yields were achieved at the stage of full maturity.

The average yield of aboveground dry matter at full maturity was 15,173 kgha^{-1} for all fertilization variants. At full maturity, dry matter of the whole plant was decreased in relation to the yield of dry matter at wax maturity by 21.2% in the control variant for and by 4.2% in the variant of manuring + NPK. Dry matter was increased in all the other fertilization variants.

At the stage of full maturity, the amount of total accumulated dry matter was decreased, evidently due to leaf atrophy and shedding.

CONCLUSIONS

Based on the results obtained in the two-year experiments, the following conclusions were drawn.

— Dynamics of dry matter accumulation depended on stage of plant development and mineral fertilization variant.

The smallest increment in dry matter in relation to dry matter in full maturity, 7.22%, was obtained at the seven-leaf stage.

— The smallest increment in dry matter for all development stages was in the control variant. Highest increments were obtained in the variant of manured two-crop rotation + NPK and the variant of manuring + NPK in the monoculture.

Highest amounts of dry matter were formed in the period from emergence to silking. In the control variant, 72% of the total yield were formed during that period. In the fertilized variants in the monoculture and the variant of manured two-crop rotation + NPK, the respective percentages were 61.0%—65.6% and 50.4%.

During the silk stage, dry matter yields in stalks and leaves were increasing, while in the period from silking to full maturity the mass of stalks and leaves decreased.

— At the stage of wax maturity, highest dry matter yields were formed in the control variant and the variant of manuring + NPK. In the other variants, highest dry matter yields were achieved at the stage of full maturity.

— Grain dry matter yield kept increasing from the stage of wax maturity to full maturity, except in the control variant where it decreased by 4.9%. Grain dry mass was increased in the NPK variant by 40.4%, in stover + NPK variant by 30% and in manuring + NPK variant by 24.7%. The highest grain dry weight increase was registered in the two-crop rotation — 41.6%.

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ДИНАМИКА СИНТЕЗЕ СУВЕ МАТЕРИЈЕ ТОКОМ РАЗВИЋА КУКУРУЗА

Драгана С. Латковић, Љубинко Ђ. Старчевић, Бранко Ј. Маринковић
Пољопривредни факултет, Нови Сад, Трг Доситеја Обрадовића 8,
21000 Нови Сад, Србија

Резиме

На вишегодишњем стационарном пољском огледу извршена су испитивања утицаја минералних и комбинације минералних и органских хранива на динамику образовања вегетативне масе код кукуруза. Динамика је праћена по фенолошким фазама и по биљним деловима.

Добијени резултати указују да се динамика накупљања суве материје мењала у зависности од фазе развића и варијанте исхране. Најмањи прираст суве материје у свим фазама испитивања био је на контролној варијанти, а највећи на варијантама где се уз минерална хранива примењивао и стајњак. У фази седам листова утврђен је најмањи прираст суве материје и он је износио 7.22% у односу на масу суве материје у пуној зрелости.