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101

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CONTENTS
САДРЖАЈ

ARTICLES AND TREATISES
ЧЛАНЦИ И РАСПРАВЕ

Зборник Матице српске за природне науке издаје Матица српска
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THERMOPHYSICAL AND ELECTRIC PROPERTIES OF ELEMENTS OF THE V A GROUP MEASURED BY MILLISECOND PULSE CALORIMETRY

ABSTRACT: In the early eighties a variant of millisecond resolution pulse calorimetry was introduced at the Institute of Nuclear Sciences „Vinča" for studying specific heat and electric resistivity of solid electric conductors. Over a period of years the method was developed to enable measurements between room temperature and 2500 K, and was adapted to measure simultaneously hemispherical total emissivity and normal spectral emissivity on the same specimens (above 1300 K). This paper reports the results obtained in a study of pure elements of the V A group: vanadium, niobium and tantalum, with the main objective to provide reliable data on their thermophysical properties in widest temperature range, contributing thus to establishing their internationally recommended functions. The obtained and available literature data are presented, and suitability of these metals as thermophysical property standard reference material candidates is discussed.

KEY WORDS: electric resistivity; elements of group V A; hemispherical total emissivity; normal spectral emissivity; specific heat, standard reference materials

1. INTRODUCTION

Results presented in this paper are a part of the ongoing study of thermal and electric properties of refractory metals conducted at the Vinča Institute of Nuclear Sciences. Beside elements of the V A group, this study involves tungsten, molybdenum and titanium.

Information on these properties in databases consist mainly of medium temperature data obtained by drop calorimetry via enthalpy measurements (to about 1300 K), and the high temperature data generated by pyrometric subsecond pulse calorimetry, starting above 1500 K (Cezairliyan, 1971a; Righini et al., 1972; Pottlacher et al., 1987). Our measurements, due to wide temperature coverage of the applied contact thermometry pulse calorimetry ranging room temperature to 2500 K (Dobrosavljević and Maglić, 1989) represent more than a link between them, providing also reliable data in the bordering ranges of other techniques, which are always characterized by lesser accuracy.

2. EXPERIMENTAL

2.1 *Method*

Modern millisecond resolution pulse calorimetry was developed at the US National Bureau of Standards in the late sixties, which was made possible by the development of new equipment for acquisition and computer processing of experimental data (Cezairliyan, 197la). To avoid problems of measuring contact temperature in a conductor which is a site of heavy current flow, this method was limited to contactless thermometry (pyrometry) range, i.e. temperatures above 1500 K. In the early eighties these problems were solved at the Institute of Nuclear Sciences „Vinča", resulting in a variant of the millisecond resolution pulse calorimetry which could be used from room temperature to extremes of thermocouple thermometry (Dobrosavljević and Maglić, 1989).

The applied method consists of fast resistive heating of electrically conducting specimen in a form of wire typically 2 mm in diameter and 200 mm long, to a predetermined maximum temperature. It involves parallel measurement of its temperature, voltage drop along the specimen effective zone and the current through specimen. For measuring temperature, three S type- or WRe 5%/WRe 26% combination thermocouples (diameter 0.05, 0.75 or 0.1 mm) are welded intrinsically in the central, effective zone (middle of the specimen), at 10 to 20 mm separations. The central thermocouple measures specimen temperature, and the other two monitor temperature uniformity within the effective zone, their P legs serving also as potential leads for measuring voltage drop along it. The procedure for compensating effects of heavy current flow through the specimen on contact thermometry is presented in detail in Dobrosavljević and Maglić (1989).

DC pulses from two heavy 12 V batteries, which are connected in series or in parallel, supply 250—600 A or 500—900 A, giving, depending on the material, heating rates of about 1000 to 4000 K s^{-1}. Measurements are performed in vacuum of 10^{-3} Pa or better to protect the specimen from oxidation and heat exchange by convection and conduction (gas phase). A PC controls the experiment and it is used for real-time acquisition of data and their subsequent processing. Data on the current, voltage drop across the effective zone and the thermocouple emf are collected during specimen heating lasting 500—1500 ms, and during the initial part of the cooling period. Several thousand data points are collected per run, yielding 500—1000 values of specific heat and electric resistivity in the whole temperature range.

Specific heat capacity, c_p, is computed from the balance of energy

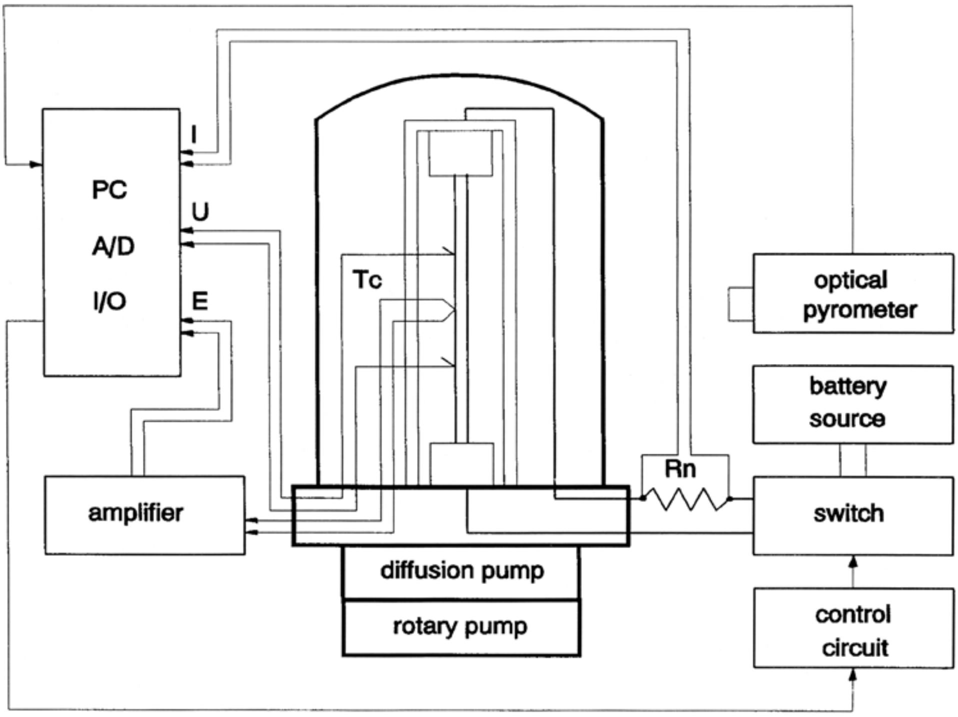

Fig. 1 — Schematics of the apparatus

$$c_p \quad \frac{UI \quad P_r}{m \,\frac{dT}{dt}\,_h} \tag{1}$$

Electric resistivity, p, follows from Ohm's law

$$r \quad \frac{US}{IL_e} \tag{2}$$

where U is the voltage drop across central, effective length, L_e, between potential leads; I is the current; P_r is the radiative power loss from the measurement zone, m is the mass of the effective specimen volume; $(dT/dt)_h$ is the heating rate at a given temperature; and S is the specimen cross section.

The radiative power loss, P_r, in (1) is given by

$$P_r \quad \varepsilon_t \sigma A\, T^4 \quad T_0^4\,) \tag{3}$$

where T_0 and T are absolute temperatures of the ambient and the specimen, respectively, A is the specimen surface area, σ is the Stefan-Boltzmann constant, and ε_t is the hemispherical total emisivity. The latter is computed at different temperatures from experimental data obtained in heating and from initial part of the cooling period of experiments ending at these temperatures, from expression

$$\varepsilon_t \quad \frac{UI}{\sigma A(T^4 \quad T_0^4)\ 1 \quad \frac{\frac{dT}{dt}\ {}_h}{\frac{dT}{dt}\ {}_c}} \tag{4}$$

Data collected during experiments with simultaneous pyrometric measurements enable determination of normal spectral emissivity. It is computed from

$$\varepsilon_\lambda \quad e^{\frac{c_2}{\lambda}\ \frac{1}{T_b}\ \frac{1}{T}} \tag{5}$$

where T is the specimen absolute temperature, T_b is the specimen brightness temperature, and c_2 is the second radiation constant. Experimental procedure is described in detail in Maglić et al. (1995/1996).

2.2 *Measurement uncertainties*

Estimation of maximum uncertainties in the specific heat and electric resistivity measurements by this method are given in Dobrosavljević and Maglić (1989), and they amount to 3% and 1%, respectively. These maximum uncertainty values apply at the upper and the lowest limits of the measurement range (Maglić et al., 1995/1996), i.e. about 150 K toward ends of the measurement range. Uncertainty is usually lower in the region between these extremes.

2.3 *Specimens*

All specimens were supplied by Goodfellow, UK, in the form of rods with nominal dimensions 2 mm in diameter and 200 mm in length. After the tantalum specimen broke in two halves, measurements were performed with the length of 110 mm. The vanadium specimen had nominal purity of 99.8%, niobium better than 99.92% and tantalum 99.91%. Typical analyses of these three metals are given in Stanimirović et al. (1999), Maglić et al. (1994) and Milošević et al. (1999), respectively. All specimens were measured as received, without additional thermal treatment.

Upper temperature limits were 1900 K for vanadium, 2500 K for niobium, and 2300 K for tantalum.

3. RESULTS

In order to increase statistical significance of final specific heat and electric resistivity functions, measurements of each material were effected in a series of experiments. Number of experiments was limited by the number of available specimens, and their deterioration in experimenting. All experiments were eventually fitted by smooth functions of specific heat and electric resistivity. The maximum temperature in different experiments varied, some of them approaching respective melting points, however, care was taken that softening should not affect original specimen geometry. In absence of reliable information on thermal expansion of the three investigated metals, data obtained for specific heat and electric resistivity were not corrected for this effect.

3.1. *Vanadium*

Specific heat and electric resistivity of vanadium were measured in seven experiments in which maximum temperatures ranged between 1898 K and 1933 K. Each of these seven experiments was represented by smooth functions of specific heat and electric resistivity. Deviations of specific heat data in individual experiments from corresponding final fitted functions were about ±1% at 400 and 1700 K, and about ±0.5% in the middle of the range. Similar, somewhat smaller deviations were observed in electric resistivity.

3.1.1. Specific Heat

The final fit representing specific heat from all experiments is given by

$$c_p \quad 467.66045 \quad 0.16172xT \quad 1.06976x10^{4}T^{2} \quad 5.31187x10^{8}T^{3} \tag{6}$$

Figure 2 presents our specific heat data, together with the literature low-temperature data of Bieganski and Stalinski (1961) and Anderson (1936), and the data above room temperature of Fieldhouse and Lang (1961), Golutvin and Kozlovskaya (1962), Bendick and Pepperhoff (1982), Jaeger and Veenstra (1934), and Cezairlyian et al. (1974). Two low-temperature data sets which both were obtained by adiabatic calorimetry extend to 340 K, and are in perfect agreement with each other (only data points above 160 K are contained in Figure 2). Our results join them at their maximum temperature. The Bendick and Pepperhoff (1982) data obtained with high-temperature adiabatic calorimetry at their lowest limit (335 K) lie somewhat lower, joining ours in the range between 367 K and 1125 K. Above 1125 K they start to lag, as their maximum temperature of 1690 K difference reaches 6.3%. Figure 2 does not show all individual data points of Bendick and Pepperhoff as their density might overcrowd the diagram, so only values at intervals of about 20 to 30 K are presented. The function of Cezairliyan et al. (1974) which extends from 1500

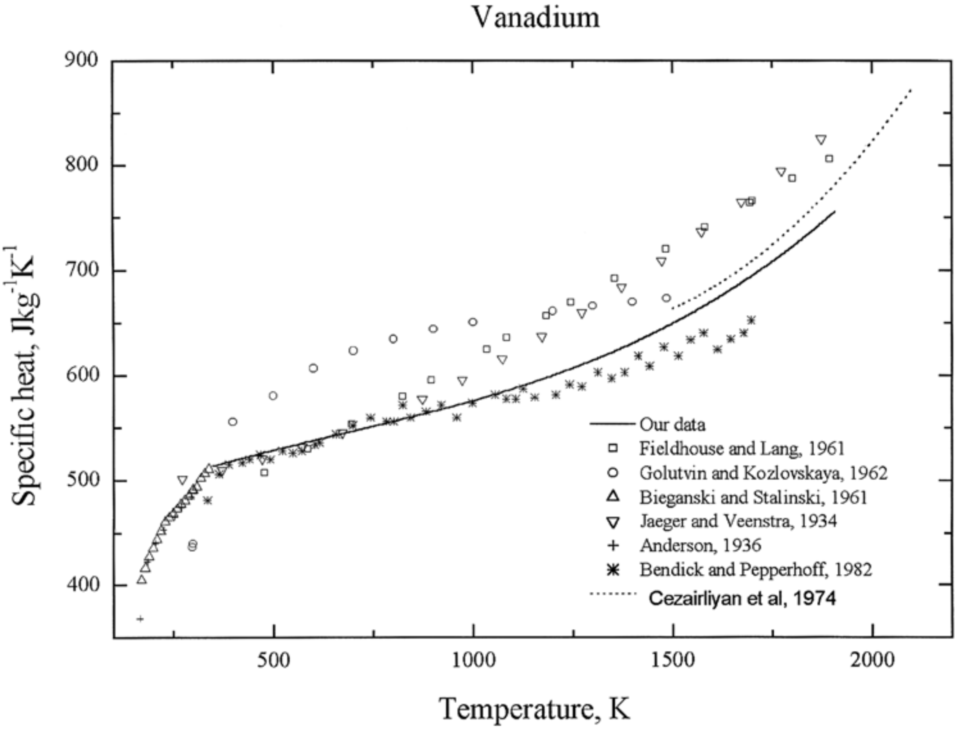

Fig. 2 — Specific heat of vanadium

to 2100 K is identical with ours, lying less than 2% above. Data sets of Jaeger and Veenstra (1934) and Fieldhouse and Lang (1961) computed from enthalpy measured by drop (metal-block) calorimetry agree with our results at lower temperatures. With increase of temperature the former follows mild parabolic, and the latter linear law. Both of them reach their maximum values at 1880 K, about 10% above our function. Data of Golutvin and Kozlovskaya (1962) (also obtained by metal-block drop calorimetry) deviate from all others.

3.1.2. Electric Resistivity

Figure 3 contains available electric resistivity data, which include our results represented by function

$$\rho \quad 3\,44821x10^{\,8} \quad 7.03044x10^{\,10}T \quad 8.84268x10^{\,14}T^{2} \qquad (7)$$

and the data of Cezairliyan et al. (1974), Peletskii (1978) and Binkele (1986). In the diagram are also included.

Comparison between electric resistivity provided by the manufacturer amounting to 19.6 μΩ·cm at 373.15 K, and our measurements of 21.3 μΩ·cm

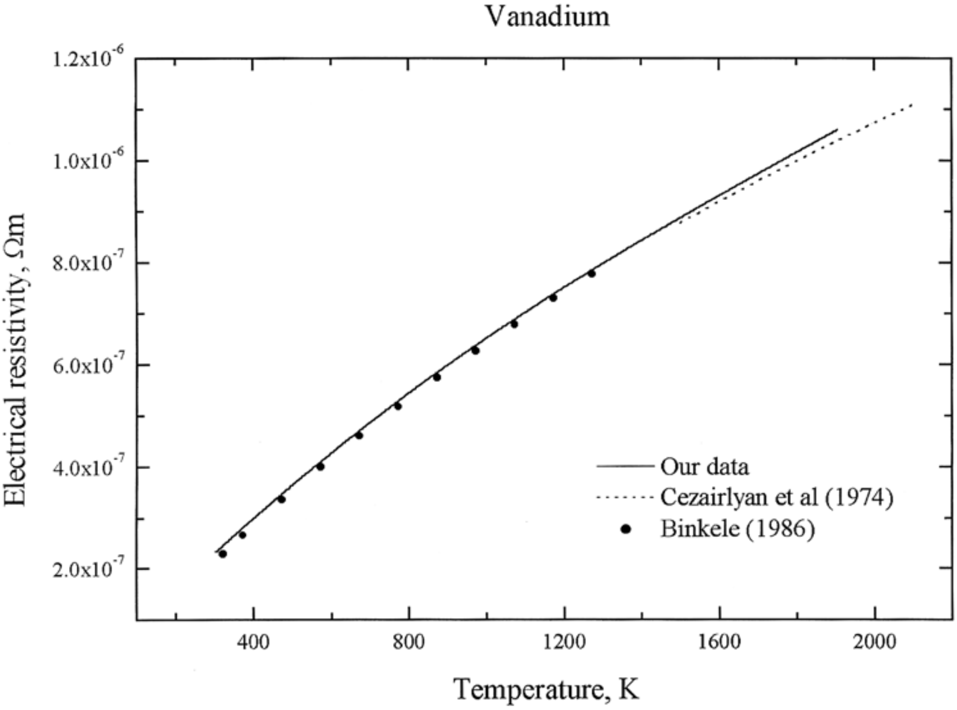

Fig. 3 — Electric resistivity of vanadium

made at the same temperature before pulse experiments with stationary state four-probe-current-reversal method (Maglić et al., 1995/1996) indicates that the specimen was not initially in an annealed state. Furthermore, the difference of 3% in our stationary four-probe measurements before the first and after the last of the pulse experiments suggests gradual increase of room temperature electric resistivity due to comparatively high cooling rates inducing changes in the metal structure. Results of Binkele (1986) for vanadium purchased from the same manufacturer with the same declared purity, lie about 7% below our values at 300 K, this difference to be reduced to 1% at 1300 K. Data of Peletskii (1978) at room temperature lie about 7% below these of Binkele (1986), this difference to be reduced to 2% at 1300 K. Data of Cezairliyan et al. (1974), which start from 1500 K, lie close to our and Peletskii's data.

3.2. *Niobium*

With the niobium specimen, a total of 32 experiments were performed, in which maximum temperatures, heating rates, thermocouple types and thermocouple wire diameters varied. Twenty-four experiments in which Pt 10% Rh/Pt thermocouple were used confined measurements to the range below 1800 K, and 8 with W 5% Re/W 26% Re thermocouple exceeded 1800 K.

For final processing, 9 experimental data sets were taken, 6 of them obtained with PtRh type and 3 with WRe thermocouples. Selection of data sets was based on the quality of primary information contained in their temperature records, primarily low noise.

3.2.1. Specific heat

Nine individual data sets with their maximum temperatures between 1300 K and 2500 K were averaged by cubic spline fits, to be represented by an interpolated specific heat function

$$c_p \quad 267.099 \quad 2.69165x10^{2}T \quad 6.72079x10^{7}T^{2} \quad 7.28962x10^{9}T^{3} \qquad (8)$$

which is presented in Figure 4. Individual data sets, which are not shown in the diagram, were confined within a zone ±1.7% at widest, the biggest difference being at lowest temperatures. Between 1000 and 2000 K these margins were about ±1%.

Beside this function, Figure 4 contains medium temperature range data of Novikov, Roschupkin and Mozgovoi (1981); widest temperature range data of Kirillin, Sheindlin and Chekhovskoi (1965) and

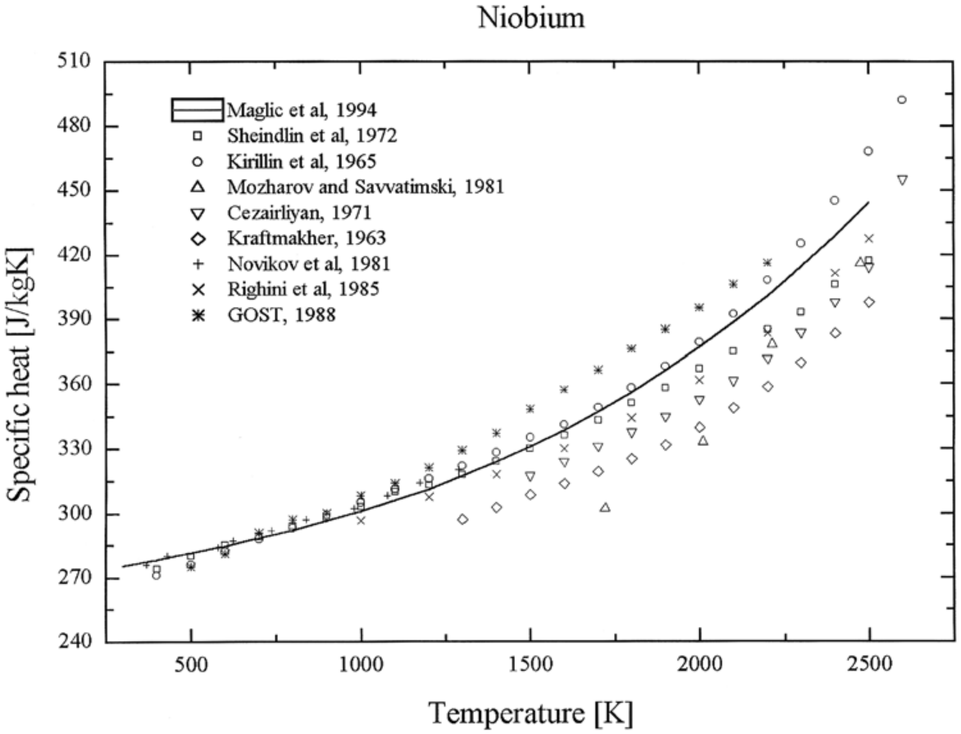

Fig. 4 — Specific heat of niobium

Sheindlin, Berezin and Chekhovskoi (1972); and the high temperature data of Kraftmakher (1963), Cezairliyan (1971b), Mozharov and Savvatimskii (1981) and Righini, Roberts and Roso (1985). Between 500K and 1800K our results show good agreement with these of Novikov et al. (1981), Kirillin et al. (1965) and Sheindlin et al. (1972), of which the first used adiabatic calorimetry and the other two levitation drop calorimetry. Agreement is also good with Righini et al. (1985) high temperature pulse calorimetry data in this range. Above 1800 K our results lie between these of Kirillin et al. (1965) and Sheindlin et al. (1965), somewhat closer to the former. Below them are data of Righini et al. (1985) lying about 4%, and Cezairliyan (1971b) 5 to 7% lower. After Kraftmakher (1963) data obtained with equivalent-impedance modulation technique are corrected, they agree with the data of Cezairliyan within 1%. Like Righini et al. (1985), Cezairliyan (1971b) used the high-temperature (pyrometric) variant of pulse calorimetry.

According to communication from the author (Kraftmakher, 1994), relatively low position of his specific heat function results from using Reimann and Grant (1936) values for temperature derivative of electric resistivity, dp/dT in computing his specific heat data. Namely, in the method he applied, C/(dρ/dT) values are measured directly. If instead of Reimann and Grant (1936) much more recent data of Righini et al. (1985) are used, reported specific heat values of Kraftmakher would shift upward between 4 and 6%.

Figure 4 also contains data of Mozharov and Savvatimskii (1981) going to very high temperature obtained by the exploding wire technique. Two lowest data points in this set evidently belong to the lower extreme in their temperature measurement range, and should not be taken with full significance. Above 2200 K they join data of Cezairliyan (1971b), both sets joining function of Sheindlin et al. (1972) at about 2500 K. The Russian Standard Reference Data for specific heat of niobium (GSSSD, 1988) are also presented.

3.2.2 Electric resistivity

Deviation of individual points from smoothed curves obtained by spline-fit averaging did not exceed ±0.1%. The final electric resistivity function

$$\rho \quad 5.63406x10^{\ 4}T \quad 1.98177x10^{\ 7}T^{2} \quad 6.26790x10^{\ 11}T^{3} \quad 7.70301x10^{\ 15}T^{4} \qquad (9)$$

is presented in Figure 5 and it represents an average of 9 measurements, which all fall within 0.67% margins. After the final pulse experiment, the specimen electric resistivity was measured using stationary four-probe-with-current-reversal technique. It amounted to 0.1506 μΩm at 293 K, comparing well with 0.1497 μΩm extrapolated from function (9) to the same temperature. Literature data for 293 K range from 0.146 to 0.169 μΩm (Mozharov and Savvatimskii, 1981).

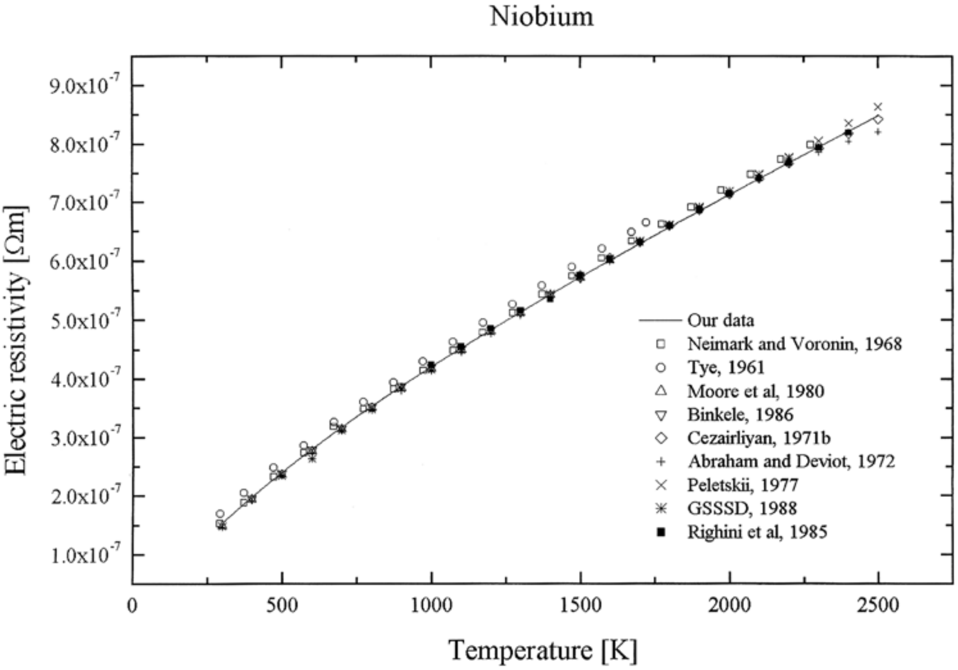

Fig. 5 — Electric resistivity of niobium

Figure 5 presents also data of Righini et al. (1985), Cezairliyan (1971b), Tye (1961), Neimark and Voronin (1968), Abraham and Deviot (1972), Peletskii (1977), Moore et al. (1980) and Binkele (1986). The Abraham and Deviot data refer to high purity niobium (1972), and the GSSSD (1988) data points represent the data recommended for specific heat of niobium (1977).

Agreement between our results and these of Righini et al. (1985), Binkele (1986), GSSSD (1988) and Cezairliyan (1971b) is remarkable. Data of Neimark and Voronin (1968) exceed the former group by about 1.5%, and these of Tye (1961) are systematically higher, between 9 to 5% following increase of temperature.

3.3. *Tantalum*

A total of 40 experiments were made on the tantalum specimen. With heating rates typical for this group of metals presence of axial heat losses was registered, so they were increased until during experiment temperature stayed uniform along the whole effective length. This limited the length of experiments to 1000 ms. For the two specimen lengths, 200 and 110 mm, the effective specimen lengths were 30 mm and 20 mm, respectively. The experiments covered the range between room temperature and about 2300 K.

Like in the case of vanadium and niobium, individual measurements resulted in continuous sets of specific heat and electric resistivity data, covering the whole, or a part of the above temperature range.

3.3.1. Specific Heat

Compared with the final function obtained by the least squares fit

$$c_p \quad 134.64 \quad 2.7981x10\ ^{2}T \quad 1.5971x10\ ^{5}T^{2} \quad 5.8259x10\ ^{9}T^{3} \tag{10}$$

individual specific heat measurements were within ±3% margins. At about 2300 K their scatter increased to ±5%.

According to the Dulong-Petit law, above the Debye temperature which for tantalum lies at about 230 K, specific heat should enter a range of saturation changing magnitude within next 2000 K by mere 30%. At elevated temperatures, however, our experimental results exceeded such prediction, adding an upswing of specific heat function above 1200 K. This was observed also by Taylor and Finch (1964), Lowenthal (1963) and Rasor and McClelland (1959). Results of Cezairliyan et al. (1971) starting above 1900 K also join the set. Lowenthal (1963) and Rasor and McClelland (1959) attributed this upswing to electronic contribution to specific heat, Rasor and McClelland (1959) also to thermal formation of lattice imperfections at high temperatures, and Taylor and Finch (1964) to other possible causes. Results of other authors investigating vanadium, niobium and other refractory metals, as well as our measurements of molybdenum (Maglić et al., 1997), confirm such a behaviour. In his treatise on vacancy formation in niobium, Kraftmakher (1963) attributed such a positive contribution of specific heat to this phenomenon.

Figure 6 compares our results with 8 available sets of literature data, agreement among them depending on the temperature range. In the range to 700 K our results were about 3% above Taylor and Finch (1964), Lehman (1970) and Kulish and Philippov (1978), and about 2% below these of Rasor and McClelland (1959). Data of Sterrett (1970) cover only the narrow lower part of the medium temperature region, their mean value laying on our curve. Above 700 K, agreement is fair with these of Taylor and Finch (1964), Lowenthal (1963), Rasor and McClelland (1959) and Cezairliyan et al. (1971c). Above 2000 K, our and Taylor and Finch (1964) results deviate somewhat higher to exceed the former three by 2.2% at 2300 K. Except for linear function of Hoch and Johnston (1961), which starts from our function at 1300 K and changes little with temperature, all results fall within a belt ±5% wide. Anomalous dependence of Hoch and Johnston (1961) might be attributed to the type of polynomial used to interpret their high-temperature enthalpy data. If instead of second order polynomial another type had been chosen, its character would have been different.

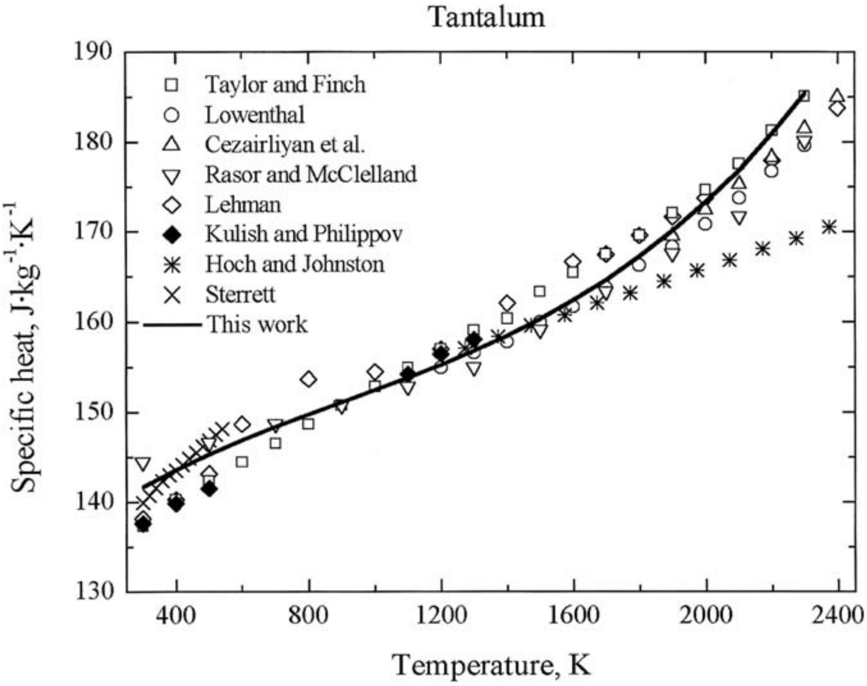

Fig. 6 — Specific heat of tantalum

It is interesting that various specific data discussed in the foregoing have been obtained with very different measurement techniques. They include three variants of thermometry in the pulse heating method: thermocouple in our study, high-speed pyrometry in Cezairliyan et al. (1971c), and specimen resistance in Taylor and Finch (1964) and Rasor and Clelland (1959). Lowenthal (1963) and Kulish and Philippov (1978) used two different methods based on temperature oscillations. The method of mixtures was used by Sterrett (1970) and Hoch and Johnston (1961), the latter being high-temperature variant of vacuum drop calorimetry.

3.3.2. Electric Resistivity

Individual electric resistivity data sets deviated ±3% at 300 K and about ±2% in the rest of the range from the final single function

$$\rho \quad 1.03x10^{8} \quad 5.1923x10^{10}T \quad 6.3911x10^{14}T^{2} \quad 5.1236x10^{18}T^{3} \qquad (11)$$

In Figure 7 our results are compared with the data of Tye (1961), Neimark and Voronin (1968), Binkele (1985), Cezairliyan et al. (1971), Denman (1969) and Taylor and Finch (1963). All results except these of Neimark and Voronin (1968) start from the same ori-

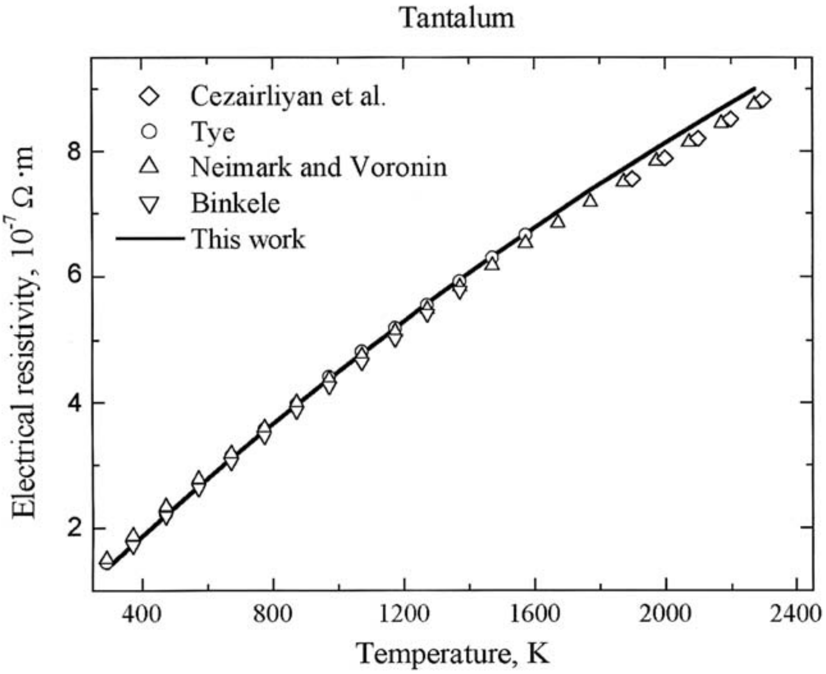

Fig. 7 — Electric resistivity of tantalum

gin near room temperature, to begin to split above 1200 K. Data of Tye (1961) and our function follow a somewhat steeper function compared with the other, which join Neimark and Voronin (1968) data. At room temperature, the latter are higher positioned than the rest. At 2300 K the difference between our function and the group amounts to 2.5%.

Before the first and after the last pulse experiment, room temperature electric resistivity was measured by the stationary state four-probe-current-reversal method.

4. DISCUSSION

In analysing reliability of data of various authors, primary weight was put on experimental methods used for their obtaining in different temperature regions. In the region below room temperature adiabatic calorimetry provides very reliable results, as illustrated by the low-temperature data of Bieganski and Stalinski (1961) and Anderson (1936). Above room temperature use of particular methods may lead to systematic errors, either arising from their inadequacy for studying particular phenomena or from incorrect interpretation of measured data.

Experiments using method of mixtures give as primary outcome enthalpy data, which must be sufficiently numerous for adequate fitting with polynomial which will be differentiated to give specific heat function. The minimum number of enthalpy data is taken to be 10, but much more are necessary for better accuracy of specific heat results, implying that temperature interval over which enthalpy measurements are made has to be sufficiently large. Accuracy of enthalpy data is further complicated by uncertainty in determining initial specimen temperature before it has been dropped. It is usually established by measuring temperature of the furnace in which specimen is held assuming they are identical, what does not have to be the case. Selection of polynomials for interpretation of enthalpy data introduces an additional difficulty, as distribution of enthalpy data (in absence of singularities) usually follows a general tendency similar to parabolic. Therefore, in order to identify delicate information contained in enthalpy vs. temperature function many data points are needed, and for selecting proper interpolating function, much experience and knowledge of the nature of specific heat is necessary (Douglas and King, 1968). Having above in mind, the levitation calorimetry should be most reliable of all variants of method of mixture, as it involves large temperature intervals and many data points and the sample temperature can be measured directly with pyrometer (Kirillin et al., 1965; Sheindlin et al., 1972).

As far as adiabatic calorimetry is concerned, its reliability decreases with temperature increase, predominantly due to difficulties in maintaining adiabatic conditions between the specimen and adiabatic shields under intensive radiation heat exchange.

Pulse calorimetry should offer most, as it avoids the pitfalls of long exposure of specimen to elevated temperatures and undesirable or uncontrollable heat exchange. The original variant based on fast pyrometric temperature measurement (Cezairliyan, 1971a) has given many valuable results in terms of specific heat, heats of fusion, and melting points of high temperature materials. Its deficiencies are that the lowest operating temperature is limited to 1500 K, and that thermal expansion of the specimen during experiment is blocked. In order to maintain position of the aperture in the specimen representing black-body cavity, the specimen has to be clamped within firm, massive sample holder. A similar apparatus operates at the IMGC[1] (Righini et al., 1972) with an advantage of having lower operating limit of 1000 K.

In the contact thermometry variant we used, the main source of uncertainty is due to heavy DC current passing through the specimen during measurements. A simple way to check the quality of thermometry is by checking the accuracy of electric resistivity measurement, as the specimen electric resistivity is used to compensate for interference from the presence of the heavy DC flow. If electric resistivity/temperature function is accurately determined, specific heat data will be accurate too (Dobrosavljević and Maglić, 1989). This variant of pulse calorimetry has also proved convenient for studying phase transitions in all types of metals.

Instead of conclusions, on the basis of the presented specific heat data and in the light of the foregoing discussion, certain recommendations could be given for specific heat functions of metals of the V A group. Electric resistiv-

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[1] The Institute of Metrology „Gustavo Colonnetti”, Turin, Italy.

ity, which is in many ways easier to measure should not be considered in a similar way, as this property is extremely sensitive to the presence of minute impurities and the state in which its structure is found. So meaningful comparisons can be made only between measurements performed on specimens with the same chemical composition and in the same physical state.

Specific heat of vanadium could follow from 400 K on character of our function, to be continued from 1900 K to 2100 K according to the function of Cezairliyan et al. (1974). Junction to the low-temperature data of Bieganski and Stalinski (1961) and Anderson (1936) near room temperature should be made in accordance with the nature of specific heat in this region. Alternately, as the function of Cezairliyan et al. (1974) lies less than 2% above ours, the final curve could be fitted to pass above 1500 K between these two data sets.

To define specific heat of niobium, all data except GSSSD (1988) and the lowest two data points of Mozharov and Savvatimskii (1981) could be used. The GSSSD data are left out because they already result from another fitting procedure. The Kraftmakher (1963) experimental data should be used after correction according to author's suggestion discussed before (Kraftmakher, 1994) i.e. to coincide with the data of Righini et al. (1985). Care should be taken to join our function to the low-temperature data by a logical, smooth function.

In defining specific heat of tantalum, virtually all data could be used except these of Hoch and Johnston (1961) for reason discussed before. Care should be taken again about junction to the low-temperature data near room temperature.

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[2] THERSYST is the name of a thermophysical properties databank for solid materials developed and operated by the University of Stuttgart-IKE, Stuttgart, Germany.

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ТЕРМОФИЗИЧКЕ И ЕЛЕКТРИЧНЕ ОСОБИНЕ ЕЛЕМЕНАТА V А ГРУПЕ ПОМОЋУ МИЛИСЕКУНДНЕ ИМПУЛСНЕ КАЛОРИМЕТРИЈЕ

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Резиме

Почетком осамдесетих у Институту за Нуклеарне науке „Винча” уведена је за изучавање специфичне топлоте и електричне отпорности чврстих електричних проводника варијанта импулсне калориметрије са милисекундном резолуцијом. Током протеклог периода метода је усавршена да се може користити у мерењима од собне температуре до 2500 К, с тим да је на истим узорцима у току истих мерења омогућено да се изнад 1300 К мере и хемисферна тотална и нормална спектрална емисивност. У раду су обрађени резултати који су добијени изучавањем чистих елемената V А групе: ванадијума, ниобијума и тантала, са основним циљем да се обезбеде поуздани подаци о њиховим термофизичким особинама у најширем опсегу изнад собне температуре, дајући тиме допринос установљавању њихових међународно препоручених функција. Приказани су и добијени литературни подаци.

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ELECTRONIC SPECTRA AND CRYSTAL STRUCTURE OF 1-METHYL-3-PHENYL-6,7 DIMETHOXYISOQUINOLINE

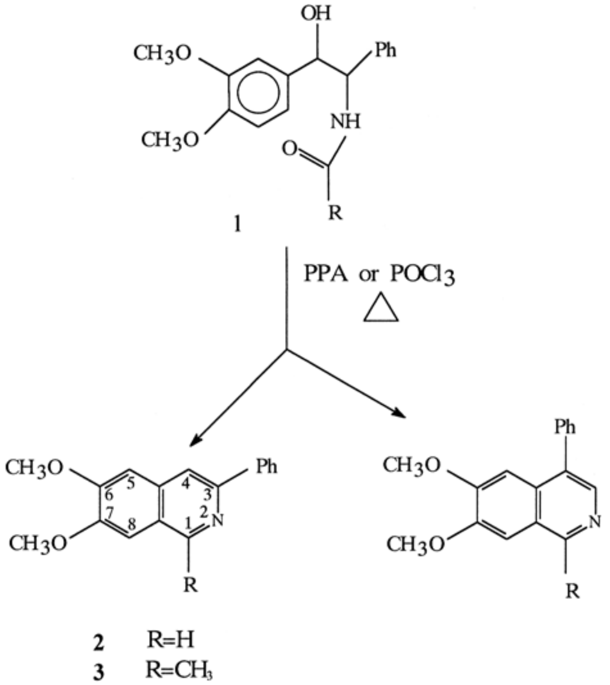
ABSTRACT: 1-methyl-3-phenyl-6,7-dimethoxyisoquinoline has been prepared by Pictet-Games reaction in order to prove its real structure i.e. to exclude the possibility of aryl migration found by Bindra et al. (1968) in the case of the cyclization of some isoquinolines. In order to achieve a better understanding of the molecule's features and to establish the possible migration of the aryl group, we have performed a study of its crystal structure by X-ray diffraction method and correlated it with absorption (at 293 K), excitation and luminescence spectra of compound in solution and fluorescence spectra in microcrystalline state at 293 and 77 K. Based on the absorption spectra it could be concluded that the phenyl substituent was bonded to position 3. The analysis of emission and excitation spectra in the solution and emission spectra in the microcrystalline state attested of large planarity of the molecule in the solid solution and crystalline environment. The great similarity of the spectra in the n-hexane solid solution and microcrystalline state led to the conclusion that no strong intermolecular interactions existed in the crystalline state. The X-ray analysis verified the formation of the desired and not of the aryl-migrated structure. The analysis of the molecule packing indicated that the planar molecules joined into pairs probably due to charge transfer. The layers formed by the pairs interacted only through van der Waal's contacts.

KEY WORDS: Electronic spectra (absorption, luminescence), isoquinoline, X-ray crystallography

INTRODUCTION

Earlier we published the synthesis of some 3-aryl-6,7-dimethoxyisoquinolines (Simon and Talpas, 1974). The intermediaries of these compounds were 2-(3,4-dimethoxyphenyl)-1-phenyl (or substituted phenyl)-N-formyl (or acetyl) ethanolamine (**1**). The cyclizations were performed according to the Pictet-Games isoquinoline synthesis and the derivatives obtained were interpreted as 3-aryl substituted ones (Scheme 1). Analogous intermediaries were cyclized by Bindra et al. (1968), to furnish 3-phenyl- (**2**), 1-methyl-3-phenyl- (**3**) and 1-methyl-3-phenyl-6,7-methylenedioxy isoquinolines. Unexpectedly they isolated a component which they claimed to be a 4-aryl derivative owing to an aryl migration. This assumption was based on the results of the analysis of the melting points of the synthesized compounds and their UV, IR and NMR spectra in solution. In order to prove our earlier interpretation, cyclizations of derivatives were repeated with polyphosphoric acid and phosphorylchloride (Scheme 1). The solvent of the crystallization was invariably ethanol, as in our case.

In order to achieve a better understanding of the molecule's features and to establish the possible migration of the aryl group, we have performed a study of its crystal structure by X-ray diffraction method and correlated it with absorption (at 293 K), excitation and luminescence spectra of compound in solution and fluorescence spectra in microcrystalline state at 293 and 77 K.



Sheme 1.

MATERIALS AND METHODS

1-methyl-3-phenyl-6,7-dimethoxyisoquinoline was synthesized by aromatization of 1-methyl-3-phenyl-3,4-dihydro-6,7-dimethoxyisoquinoline obtained by the Bischler-Napieralski ring-closure (Scheme 1). The melting point 149—151°C was in the same region as in the previous work of Simon and Talpas (1974). For spectroscopic measurements, the examined compound was recrystallized from ethanol. The solvents used were of analytical purity and the solution concentrations were 10^{-5} —10^{-2} mol dm^{-3}.

The absorption spectra were measured on a Zeiss VSU-1 spectrophotometer. The luminescence and excitation spectra were recorded on an Aminco-Bowman spectrophotofluorometer with an off-axis ellipsoidal mirror condensing system. A round quartz cuvette (ϕ 1 mm) was used for the solutions as well as for microcrystalline samples at both, room temperature and 77 K. All presented spectra were corrected for the spectral response of the equipment. The positions of the vibrational maxima were determined with experimental error of ±1 nm. The infrared spectra were registered by a Zeiss IR spectrophotometer Specord 75.

The colorless transparent single crystals, of irregular shape, dimensions 0.50 x 0.25 x 0.20 mm and very poor quality, were examined by X-ray diffraction method in air conditions. Cell parameters (Tab. 1) were determined by least-squares refinement of diffractometer angles. Intensities of reflection were recorded on a Simens R3m/V diffractometer equipped with CuK_p ($\lambda = 1.54178$ Å) radiation monochromated by highly oriented graphite crystal and using a θ scan in the range $3.0 < 2 < 110.0°$. Three standard reflections were monitored every 97 reflections with intensity variation $R_{int} = 0.043$. A total of 2836 reflections were recorded with Miller indices —1 *h* 10, —1 *k* 28 and —12 *l* 1, merged to 1831 independent (unique) reflections. Data were corrected for Lorenz and polarization effects and not for absorption.

Tab. 1. X-ray crystallographic data

Crystal data	
Empirical formula	$C_{18}H_{17}NO_2$
Color; habit	Colorless, chunk
Crystal size (mm)	0.50 x 0.25 x 0.20
Crystal system	Orthorhombic
Space group	Pbca
Unit cell dimensions	a = 9.452(2) Å b = 26.356(2) Å c = 11.782(2) Å
Volume	2935.1(8) Å^3
Z	8
Formula weight	279.3
Density (calc.)	1.264 Mg/m^3
Absorption coefficient μ	0.657 mm^{-1}
F (000)	1184
Data collection	
Temperature (K)	296
Index ranges	$-1 \leq h \leq 10$ $-1 \leq k \leq 28$ $-12 \leq l \leq 1$
Reflections collected	2836
Independent reflections	1831 (R_{int} = 4.30%)
Observed reflections	1398 ($F > 3.0\ \sigma(F)$)

Solution and refinement	
Refinement method	Full-matrix least-squares
Quantity minimized	$\Sigma w(F_o—F_c)^2$
Extinction correction	$\chi = 0.0030(10)$, where $F^* = F[1 + 0.002\chi F^2/\sin(2\theta)]^{-1/4}$
Weighing scheme	$w^{-1} = \sigma^2(F) + 0.0047F^2$
Data/restraints/parameters	1398/68/191
Final R indices (obs. data)	R = 6.72%, wR = 10.27%
R indices (all data)	R = 9.83%, wR = 14.70%
Goodness-of fit	1.25
Largest and mean Δ/σ	0.001, 0.000
Largest difference peak and hole	0.37 eÅ^{-3}, –0.33 eÅ^{-3}

The structure was solved by direct method using Siemens SHELXTL PLUS (VMS) (Sheldrick, 1991). The positions of the non-hydrogen atoms were refined anisotropically up to the final R-factors R = 0.0672 listed in Table 1. The hydrogen atoms were generated from an assumed geometry and refined in riding mode with fixed isotropic displacement parameters (U = 0.080 Å^2). Scattering factors were taken from SHELXTL. A list of structure factors and anisotropic displacement parameters for the non-hydrogen atoms and hydrogen atomic positions are available from one of the authors (A. Kapor, R. Willett) on request.

RESULTS AND DISCUSSION

Spectroscopic measurement

The absorption of 1-methyl-3-phenyl-6,7-dimethoxyisoquinoline was measured at room temperature in several solvents. The shape and position of absorption bands depended little on solvent nature. The absorption spectra in dilute n-hexane solution are presented in Figure 1 (curve 1). The three absorption bands, identified as ^1B_b, ^1L_a and ^1L_b, had maxima at 260, 298 and 339 nm and molar absorptivities ε = 4667, 1890 and 210 m^{2}mol^{-1}, respectively. The comparison with corresponding bands in isoquinoline absorption spectrum shows that ^1L_b band ($S_0 \rightarrow S_1$), although not well resolved, was red-shifted with respect to that of isoquinoline and its vibrational structure has vanished. ^1L_a band ($S_0 \rightarrow S_2$) was also red-shifted and considerably intensified. Still, the most characteristic feature of the spectrum was a large red-shift (about 7000 cm^{-1}) of ^1B_b band ($S_0 \rightarrow S_3$) with respect to isoquinoline one, which indicated the extension of the conjugated system in the longitudinal direction and strong interaction of phenyl ring with isoquinoline nucleus. This spectrum is in good correlation with absorption spectra of 2-phenylnaphtalene (Friedel and Orchin, 1951), but not with those of 1-phenylnaphthalene, where there was a steric hindrance of planarity. This led to a conclusion that the phenyl ring in the examined molecule was in position 3, as confirmed by the X-ray crystal analysis.

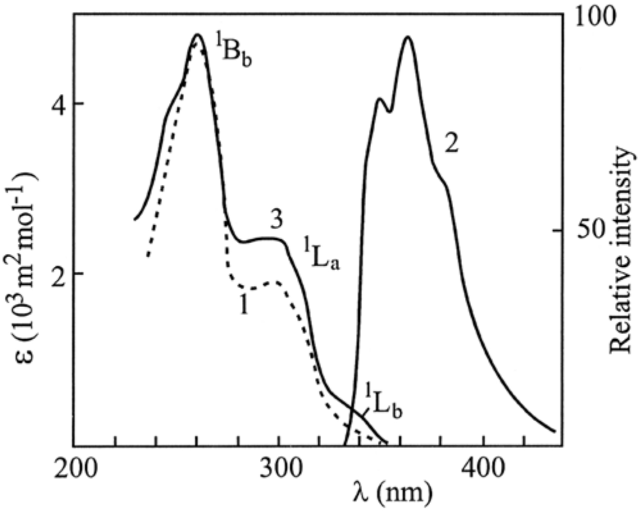


Fig. 1. — Absorption (1), fluorescence (2) and fluorescence excitation spectrum (3) in n-hexane solution ($4 \cdot 10^{-5}$ mol dm^{-3}) at 293 K

The emission and excitation spectra of the examined molecule were measured in liquid solutions at room temperature and in solid solutions at 77 K. At room temperature, the position and shape of the fluorescence spectrum depend weakly on the solvent nature and concentration. So the structureless fluorescence spectrum with maximum at about 370 nm in ethanol and dioxane stayed practically unchanged when increasing the concentration up to 10^{-2} mol/dm^3, which indicated that there was no excimer formation. In dilute n-hexane solution, a slightly structured fluorescence spectrum appeared with maximum at 364 nm (Figure 1, curve 2). The corresponding fluorescence excitation spectrum (Figure 1, curve 3) correlated well with the absorption spectrum, as in band positions so in relative intensities of the bands, which confirmed that the registered fluorescence originated from the examined compound. In ethanol glass solution at 77 K, fluorescence spectrum was decomposed into several vibrational bands with maxima at 347, 362, 379 and 402 nm and also there appeared phosphorescence in the visible region. The relative intensities of the fluorescence vibrational bands depend on concentration.

The luminescence and excitation spectra in solid n-hexane solution exhibited expressed vibrational structures (Figure 2), which indicated a high degree of planarity of the examined molecule (Berlman, 1971). However, since its structure is not rigid, due to a single bond between the isoquinoline nucleus and phenyl ring, the planarity at room temperature was disturbed and the vibrational structure was blurred. The intensity of the 0—0 fluorescence band (343 nm) decreased as the concentration increased, as a result of self-absorption. This effect was also registered in the fluorescence spectra of isoquinoline and 3-methylisoquinoline (Janić and Kawski, 1973). The fluorescence vibrational bands with maxima at 343, 360, 380 and 401 nm constituted the progression of vibration with the frequency of 1400 cm^{-1} (curve 1). Besides that, there also appeared vibrations with frequencies of 400 and 900 cm^{-1}. In the phosphorescence spectrum (curve 2), vibrational bands with maxima at 483 (0—0 transition), 502, 524, 546 and 572 nm formed the progression with the frequency of 800 cm^{-1} and there also appeared vibrations with frequencies 200, 450 and 1400 cm^{-1}. The frequency values of the basic vibrations in the ground electron state of the examined molecule obtained by luminescence spectrum agreed well (within the limits of experimental error) with those (413, 787, 893 and 1380 cm^{-1}) in the infrared spectra of the examined compound in CCl_4 solution and KBr disk, which were registered in the region above 400 cm^{-1}. The values of 200 and 450 cm^{-1} could be correlated with frequencies of 183 and 460 cm^{-1}, which appears in Raman spectrum of isoquinoline (Kawski et al., 1977).

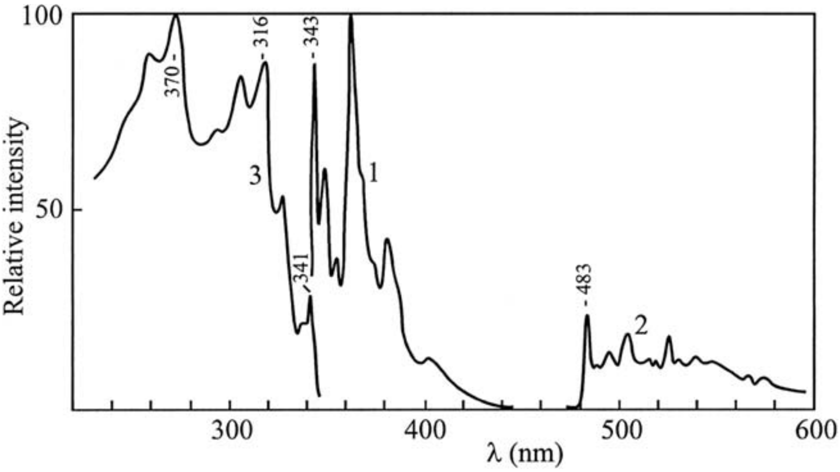


Fig. 2. — Fluorescence (1), Phosphorescence (2) and excitation (3) spectrum in n-hexane solution (4 · 10^{-5} mol dm^{-3}) at 77 K

The narrow vibronic bands in the excitation spectrum (curve 3) made possible to determine the 0—0 transitions in the individual electronic bands ^1L_b, ^1L_a and ^1B_b, which were 341, 316 and 270 cm^{-1}, respectively. The frequency of about 1400 cm^{-1} was obtained for the most intense vibration in all three electronic absorption bands of the molecule, which correlated with the values of 1390.5 and 1406.5 cm^{-1} obtained from the quasi-linear absorption spectra of isoquinoline and 3-methyl-isoquinoline, respectively (Kawski et al., 1977).

1-methyl-3-phenyl-6,7-dimethoxy-isoquinoline in microcrystalline state exhibited intensive fluorescence, but phosphorescence was not registered, even at 77K. The fluorescence spectrum was structured already at room temperature and partially at low temperature (Figure 3), with vibrational maxima very close in position to that of the molecule in solid n-hexane solution:

microcrystal	[nm]	**—**,	348,	353,	**359**,	365,	—,	**379** …
n-hexane solution	[nm]	**343**,	348,	354,	**360**,	366,	372,	**380** …

(The values in bold correspond to the vibrational bands which constitute the progression). A comparison of relative band intensities in the short-wave part of the fluorescence spectrum of the microcrystalline sample with those in n-hexane solid solution of higher concentrations, indicates the presence of intensive self-absorption in the microcrystalline state. As a result, the first short-wave vibrational band, which corresponds to 0—0 transition, was absent (see above). It was evident that the emission of the examined molecule in crystalline environment was little altered in relation to that when the molecule was in a neutral solid solvent such as n-hexane, which indicated a weak intermolecular interaction in the crystal.

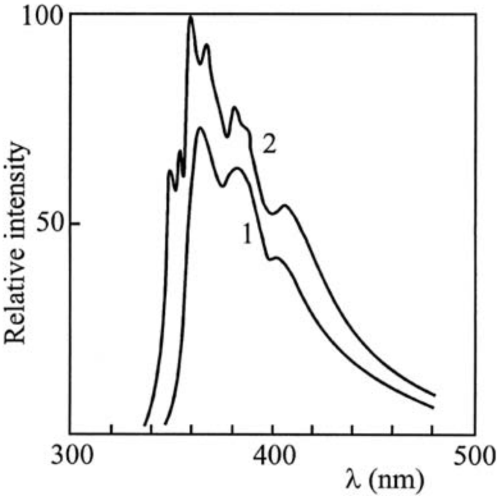


Fig. 3. — Fluorescence spectra in microcrystalline state:
(1) at 293 K and (2) at 77 K

X-ray crystal structure solution

Fractional atomic coordinates and equivalent isotropic thermal parameters for non-hydrogen atoms are given in Table 2. Bond distances and bond angles are listed in Table 3. The solid-state conformation of the molecule is given in Figure 4. The analysis of bond lengths showed good agreement with theoretical values. The length of the bond N2-C1 (1.325(4)Å) were indicative of its double character, while the other bond lengths in the isoquinoline ring indicated the broadening of the scope of π-electron system of the nitrogen atom N2 along the direction N2-C3-C4, which resulted in the shortening of the bonds N2-C3=1.371(4)Å and C3-C4=1.373(5)Å. The analysis of geometry showed that the phenyl ring was bonded in position 3 of the isoquinoline ring. The position of the phenyl ring was stabilized by intramolecular hydrogen close contacts C10-H10… N2 = 2.851(5)Å, H10… N2 = 2.556(3)Å, C10-H10… N2 = 98.1(3)° and C14-H14… C4 = 2.987(7)Å, H14… C4 = 2.731(5)Å, C14-H14… C4 = 95.8(3)°.

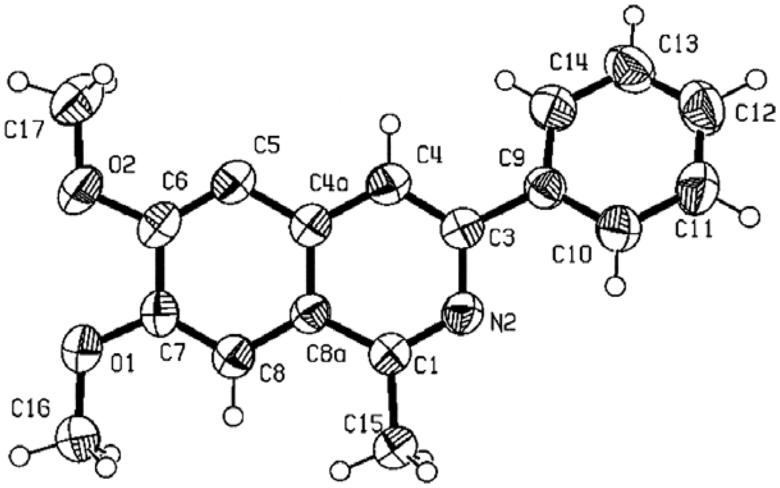


Fig. 4. — ORTEP drawing of the molecule with the non-H atom labeling scheme. The displacement ellipsoids are draw at 50% probability (Jonson et al., 1997)

Tab. 2. — Fractional atomic coordinates (x10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3)

	x	y	z	U(eq)
O (1)	794(3)	5288(1)	8898(2)	58(1)
O (2)	2409(3)	5939(1)	9853(2)	66(1)
N (1)	3063(3)	6173(1)	4481(3)	49(1)
C (1)	2293(4)	5864(1)	5118(3)	47(1)
C (3)	3905(4)	6529(1)	4995(3)	46(1)
C (4)	3994(4)	6563(2)	6156(3)	49(1)
C (4a)	3187(4)	6244(1)	6852(3)	45(1)
C (5)	3236(4)	6271(2)	8050(3)	51(1)
C (6)	2435(5)	5950(2)	8696(3)	52(1)
C (7)	1529(4)	5585(2)	8167(3)	46(1)
C (8)	1471(4)	5552(2)	7011(3)	48(1)
C (8a)	2294(4)	5879(1)	6330(3)	43(1)
C (9)	4731(4)	6857(1)	4228(3)	47(1)
C (10)	5146(4)	6685(2)	3157(3)	58(2)
C (11)	5995(5)	6980(2)	2465(4)	68(2)
C (12)	6412(5)	7453(2)	2818(4)	69(2)
C (13)	5987(5)	7635(2)	3854(4)	69(2)
C (14)	5162(5)	7338(2)	4561(4)	62(2)
C (15)	1423(5)	5480(2)	4495(3)	59(1)
C (16)	–62(4)	4898(2)	8420(4)	60(2)
C (17)	3204(5)	6325(2)	10426(4)	74(2)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

The molecule as a whole was approximately planar, except for the phenyl ring which was twisted (N2-C3-C9-C14=154.3(4)°) with respect to the rest of the molecule. Stronger thermal vibrations were noticed in the O2-CH_3 methoxy group and the peripherals of the phenyl ring.

Tab. 3. — Bond lengths (Å) and angles (°)

Bond lengths (Å)					
O1	C7	1.355(5)	O1	C16	1.424(5)
O2	C6	1.364(4)	O2	C17	1.434(6)
N2	C1	1.325(4)	N2	C3	1.371(4)
C1	C8A	1.429(5)	C1	C15	1.496(6)
C3	C4	1.373(5)	C3	C9	1.474(5)
C4	C4A	1.400(5)	C4A	C5	1.414(5)
C4A	C8A	1.420(5)	C5	C6	1.367(6)
C6	C7	1.431(7)	C7	C8	1.366(5)
C8	C8A	1.411(5)	C9	C10	1.397(5)
C9	C14	1.388(6)	C10	C11	1.383(6)
C11	C12	1.372(7)	C12	C13	1.372(7)
C13	C14	1.384(7)			

Bond angles (°)							
C7	O1	C16	117.2(3)	C6	O2	C17	116.5(3)
C1	N2	C3	119.3(3)	N2	C1	C8A	123.3(3)
N2	C1	C15	116.1(3)	C8A	C1	C15	120.6(3)
N2	C3	C4	121.3(3)	N2	C3	C9	116.0(3)
C4	C3	C9	122.7(3)	C3	C4	C4A	120.7(4)
C4	C4A	C5	122.4(3)	C4	C4A	C8A	118.5(3)
C5	C4A	C8A	119.1(3)	C4A	C5	C6	120.4(4)
O2	C6	C5	125.4(4)	O2	C6	C7	114.2(4)
C5	C6	C7	120.3(4)	O1	C7	C6	114.7(4)
O1	C7	C8	125.2(4)	C6	C7	C8	120.1(4)
C7	C8	C8A	120.4(4)	C1	C8A	C4A	116.9(3)
C1	C8A	C8	123.4(3)	C4A	C8A	C8	119.7(3)
C3	C9	C10	120.8(3)	C3	C9	C14	121.2(3)
C10	C9	C14	118.0(4)	C9	C10	C11	120.9(4)
C10	C11	C12	119.9(4)	C11	C12	C13	120.2(5)
C12	C13	C14	120.2(5)	C9	C14	C13	120.8(4)

The molecular packing into the crystalline lattice along the c-axis is shown in Figure 5. Although we did not notice any stronger intermolecular contacts (Table 4), the packing scheme showed the grouping of the molecules into pairs, probably due to charge transfer. The electron-rich side of the isoquinoline molecule combined with the opposite side of another symmetry-transformed isoquinoline molecule in the crystal unit cell. The pairs of molecules formed chains along the a-axis and these chains were stacked in a zigzag manner parallel to the ab-plane.

Tab. 4. — Selected intermolecular hydrogen close contacts

X — H...Y	X — H [Å]	H...Y [Å]	X...Y [Å]	∠X — H...Y [°]	Symmetry transformation
15-H15...O1	0.954(5)	2.535(3)	3.393(6)	149.7(1)	1/2–x, 1–y, –1/2+z
C16-H16A...O1	0.979(5)	2.466(2)	3.272(5)	140.4(3)	–x, 1–y, 2–z
C16-H16A...O2	0.970(3)	2.801(3)	3.732(5)	161.2(3)	–x, 1–y, 2–z

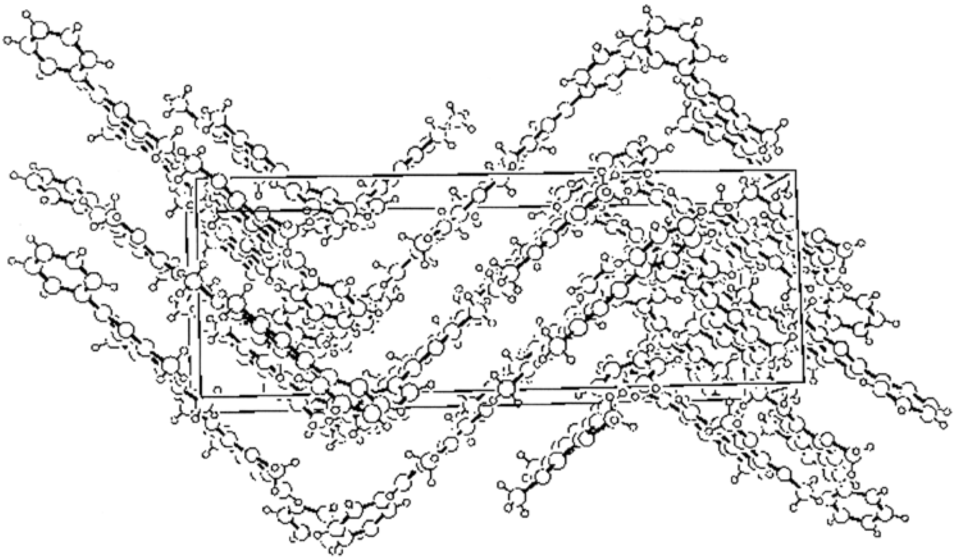


Fig. 5. — The crystal packing for the unit cell projected along the c-axis

The X-ray crystal analysis confirmed the bonding of the phenyl ring in position 3 as predicted by the analysis of the absorption spectra of the molecules in solution. It was evident that the emission of the examined molecule in crystalline environment was little altered in relation to that when molecule is incorporated in a neutral solid solvent such as n-hexane, which indicated the weak intermolecular interaction in the crystal. Weak intermolecular interaction was preserved also in the single crystal state, as confirmed by the analysis of intermolecular contacts in the crystal lattice.

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ЕЛЕКТРОНСКИ СПЕКТРИ И КРИСТАЛНА СТРУКТУРА 1-МЕТИЛ-3-ФЕНИЛ-6,7-ДИМЕТОКСИИЗОХИНОЛИНА

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Резиме

1-Метил-3-фенил-6,7-диметоксиизохинолин је синтетизован мето-дом Пиктет-Гамс реакције, с циљем да се докаже стварна структура продукта, односно да се искључи сумња у постојање миграције фенилног прстена, што је запажено раније при циклизацији неких сличних деривата изохинолина (Bindra et al. 1968). У циљу бољег упознавања карактеристика наведеног молекула и утврђивања могуће миграције ароматичног прстена, извршена је анализа кристалне и молекулске структуре методом дифракције X-зрака, а резултати су упоређени са информацијама добијеним на основу апсорпционих спектара (на 273 K), екситационих и луминисцентних спектара супстанце у раствору и флуоресцентних спектара поликристалне форме на 293 и 77 K. На основу апсорпционих спектара закључено је да је фенил група везана у положају 3 изохинолинског језгра. Анализа емисионих и ексцитационих спектара у раствору и емисионих спектара у микроскристалном стању указује на висок степен планарности молекула у чврстом раствору и кристалном окружењу. Велика сличност спектра у чврстом раствору н-хексана и микрокристалном стању наводи на закључак да у кристалном стању не постоји јача интермолекулска интеракција. Анализа помоћу X-зрака потврђује да је при синтези формирана планирана структура, без миграције фенил групе. Анализа паковања молекула у кристалну решетку показује груписање планарних молекула у парове вероватно због преноса наелектрисања између π-електронских система изохинолинског језгра. Код слојева формираних од парова молекула јавља се само Ван дер Валсова интеракција.

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MINERALOGY AND AGROCHEMSTRY OF MAGNESIUM IN SOILS OF VOJVODINA, ŠUMADIJA AND NORTHERN POMORAVLJE

ABSTRACT: Magnesium is an important element of the biosphere, hydrosphere and lithosphere. In the biosphere it is an essential macronutrient for plants, animals and humans. Magnesium deficiency in humans could be linked to the pathogenesis of cancer or it might be related to sudden cardiac deaths in areas with soft water.

Total contents Mg and Ca were determined in $HClO_4$ + HF digests by AAS method in selected soil samples, whilst IM NH_4OAc extraction was carried out to assess Mg and Ca bioavailability to plants from different soils. The mineralogy of bulk soil samples was obtained by XRD analysis, using SIMENS 2 kW diffractometer IN Co Kα radiation. The DRX Win 1.4c (1996) computer package was used to identify and quantify soil mineralogy. All data obtained were statistically analyzed by computer package STATISTICA for Windows 4.3b (1993). Raster maps showing the distribution of Mg were drawn by computer using UNIRAS subroutine.

This investigation has shown that the investigated soils have the available Mg content between 10 and 93 mg/100g, with an average of 40.8 mg/100 g, and thus could be considered as Mg sufficient for plant production. Available Mg is primarily associated with carbonate fraction (dolomite) and to a lesser extent with exchangeable (clay minerals) and silicate forms (chlorite, mica, amphibole). Total content of Mg varies between 0.20 and 1.26%, with an average of 0.603%. The Ca/Mg ratio has shown a large scale variation between 2.5:1 and 78.8:1. Only 10% of the soils have Ca/Mg ratio lower then 5:1 or optimal ratio for plant nutrition, whilst 90% of the soils were found to have the ratio above 5:1 which, in case of extremely high values, might be a limiting factor in plant growth and might influence an adequate supply of Mg to animals and humans. This investigation has emphasized a necessity of wider research in soil mineralogy and chemistry of our soils, which are considered as being sufficient in available magnesium.

KEY WORDS: soil mineralogy, magnesium, calcium, Ca/Mg ratio, distribution maps

1. INTRODUCTION

Magnesium is one of the prevalent elements in the earth's crust. It is the eighth most abundant of the elements and estimates of its content have ranged from 2.0% to 2.5%. It is an important major constituent of the biosphere, hydrosphere and nearly all rock types of the lithosphere. According to Rankama and Sahama (1950), in the upper lithosphere, magnesium is a lithophile element with biophile tendencies. Average concentrations of magnesium in the earth's crust, selected rock types, water and soils are listed (Lowton, 1955; Vinogradov, 1959; Mengel, 1979; and Ure, 1982) in Table 1.

Tab. 1 — Abundance of magnesium in various natural environments (10^4 mg/kg)

Lithosphere	2.5	Sandstone	0.9
Ultra basic rocks	20.4	Marl and clay	2.1
Basic rocks	4.6	Carbonates	4.7
Granites	0.6	Soils	0.6
Shale	1.9	Sea water	0.13

Highest magnesium contents are recorded in the earliest rocks to crystallize, particularly ultrabasic and basic rocks, peridotites, gabbros and basalts, while more acid rocks such as granites, granodiorites, trachytes and rhyolites contain only small amounts of magnesium. Shales and marls contain similar amounts of magnesium to those found in igneous rocks, while sandstones and residual sediments in general contain less magnesium. According to Rankama and Sahama (1950) and Goldschmidt (1950), the content of magnesium in sedimentary rocks varies from traces in some sandstones to 3800 mg/kg in limestone, 11000 mg/kg in clays and up to 13% in dolomites.

In the igneous silicate rocks, magnesium is present in the divalent state associated largely with the ferromagnesian and accessory minerals. Progressive leaching occurs in the process of weathering. Magnesium is removed from the zone of weathering as bicarbonate. The solubility and mobility of Mg is relatively high, irrespectively of pH and Eh conditions. The precipitation of dissolved magnesium in the form of carbonates is controlled by pH and Eh of solution and follows the precipitation of calcium. In the geochemistry of surface processes of leaching, gleying and surface organic matter accumulation, together with soil properties such as reaction — pH and redox potential — Eh, affect the distribution, form and mobility of magnesium and calcium in soil.

According to Mengel and Kirkby (1979), in areas with humid climate, average magnesium content in sandy soils is usually 0.05%, while clayey soils contain up to 0.5%. Magnesium is a constituent of more than 180 minerals although only sixty can be classed as magnesium-bearing ores. The most common naturally occurring sources of magnesium are: olivine, pyroxene, serpentine, magnesite, dolomite, brucite, as well as biotite and hornblende. It is also constituent of clay minerals such as vermiculite, illite, montmorillonite and chlorite. In some soils it is present as magnesite, $MgCO_3$, or dolomite, $CaCO_3MgCO_3$, while some soils in arid and semiarid regions may contain significant amounts of epsomite, $MgSO_4$ $7H_2O$.

Magnesium in soils occurs in water soluble, exchangeable and non-exchangeable forms. There is a constant balance between these three forms. The greatest part of magnesium is found in residual, non-exchangeable form, bound to primary or clay minerals. The other two forms water soluble and exchangeable, are the major sources of available magnesium to plants. The availability of soil magnesim to plants is controlled by the following factors: amount of available Mg, soil reaction (pH), CEC, soil texture, type of soil colloids and the ratio of magnesium to the other elements e.g. Ca and K in soil solution (Haby, 1990).

In the biosphere, magnesium is naturally a major essential macronutrient vital to both plant and animal life (Mengel and Kirkby, 1979). Chlorophyll pigment in plants is a Mg-porphyrin complex, and all enzymatic reactions in animals and man that are catalyzed by ATP require Mg as a co-factor. Oxidative phosphorylation, DNA transcription, RNA function, protein synthesis and critical cell membrane functions are all dependent upon optimal Mg concentrations (Thorton (Ed.), 1983).

Magnesium deficiency in humans has been receiving increasing attention. Several reports indicate that Ca and Mg concentrations in human tissues vary with the hardness of municipal water supplies (McMillan, 1978). These suggest an important geochemical influence. Since cardiac arrhythmias are serious manifestation of Mg deficiency, a hypothesis of sudden cardiac deaths in geographic areas with soft water (low in Mg content) might be related. Magnesium deficiency has been linked to pathogenesis of cancer both experimentally in animals and epidemiologically in man. Also, there is evidence that Mg may relate to the formation of kidney stones. According to Blondell (1980), there are some evidence that geochemical availability may relate to human tissue Mg levels, which is essential to the hypothesis that the geochemistry of Mg might relate to human disease.

MATERIAL AND METHODS

During large-scale sample collection for a project financed by the Ministry of Science and Technology, an orthogonal regular 10 x 10 km grid was used to avoid bias in site location. From the set of about 5000 samples a collection of samples from Vojvodina, Šumadija and Northern Pomoravlje was taken to represent the most important soils. Selection was restricted to the samples from the arable layer with 0—25 cm depth.

After collection, samples were air-dried and sieved to pass through a 2 mm sieve. A 50 g subsample of soil was obtained by conning-and-quartering and then ground, to less then 150 mesh, in an all-agate planetary ball-mill. Total concentrations of Mg and Ca in $HClO_4$ + HF digests were determined by AAS analysis. The 1M ammonium acetate extraction was carried out to assess the magnesium and calcium bioavailability to plants from different soils.

The mineralogy of bulk soil samples was obtained by X-ray diffraction analysis (XRD) of random-packed powder mounts, after they were ground and freeze-dried. XRD patterns were obtained by using SIMENS 2 kW diffractometer, with iron filtered Co Kα radiation. DRX Win 1.4c (1996) computer package was used to identify and quantify soil mineralogy. Characterization of minerals in soils was carried out according to the method described in Brindly and Brown (Ed.) (1980) and Klute (Ed.) (1986). All data obtained were statistically analyzed by computer package STATISTICA for Windows 4.3b (1993).

The classes used to represent the data on the map were chosen from the box and wiskers analysis. Thus, the map has 5 classes and a raster map showing the distribution of magnesium was drawn by computer using UNIRAS subroutine.

RESULTS AND DISCUSSION

Mineralogy of the soils

The mineralogical composition of the bulk samples of the investigated soils is complex and it is presented in Table 1. The predominance of quartz, mica, associated with altered feldspars (plagioclase and orthoclase), carbonate (calcite and dolomite), and minor to trace amounts of chlorite, clay minerals, hornblende and rare goethite and talc may indicate the mineralogy and variety of parent rocks e. g. loess, schists, flisch sediments and sandstones. Magnetite, ilmenite and other accessory minerals (garnets, epidote, apatite, pyroxene) are found in heavy mineral fractions. Variation in quartz content might reflect weathering processes that have developed during pedogenesis. The soils with stronger weathering processes (distric cambisols and luvisols) contain higher quartz content in comparison to less weathered soils such as fluvisols, rankers and regosols, on the same and similar parent rocks.

It is evident that the soils from Vojvodina (chernozem, halomorphic soils, semigleys, humogleys and eugleys) are more abundant in micas and chlorite compared with the southern soils of Šumadija and Pomoravlje, where eutric and distric cambisols and luvisols occur, which contain less mica and chlorite. Further comparison of the northern to southern areas has shown that northern of Vojvodina (chernozems and semigleys) comprise up to twice higher carbonate contents then southern areas or Šumadija soils (distric cambisols, luvisols and pseudogleys). In northern areas, dolomite dominates over calcite in semigleys and cernozems, while calcite is more abundant then dolomite in fluvisols, humo- and eugleys. In the southern areas in some eutric cambisols and some luvisols calcite is only mineral present in the bulk soil, while in others soil carbonates are absent due to complete leaching as in more acidic distric cambisols. This trend could be only explained by preferencial leaching of carbonates, particularly dolomite, in the areas with high annual precipitation.

The major weathering process in the soil in a wet, slightly acidic environment involved the disappearance of micas and chlorite from the surface horizons and their conversion to expandible minerals such as vermiculite and smectite. The degree of mica and chlorite alteration has correlated with the development of brownized and humous varieties of the soils.

Tab. 2 — Average mineral composition (%) of the bulk samples of the investigated soils

	Quartz	Plagio-clase	Ortho-clase	Chlo-rite	Mica + Illite	Smec-tite + Verm.	Mixed Layers	Kaoli-nite	Calcite	Dolo-mite	Goetite
Chernozem	53.8	8.7	0.7	6.0	19.0	0.9	0.3	1.0	4.5	4.9	0.2
Halomorphic soils	57.3	10.4	0.7	4.7	20.6	0.5	0.4	1.1	1.2	2.3	0.2
Semigley	52.5	8.5	0.6	5.4	19.8	1.8	0.2	1.5	4.3	5.1	0.2
Humo- + Eugley	52.9	11.2	0.7	5.3	21.7	2.6	0.3	1.7	1.7	1.5	0.3
Fluvisol	51.2	10.4	0.6	5.7	22.4	3.3	0.2	2.2	2.3	1.7	0.2
Pseudogley	67.6	6.4	0.5	4.6	16.5	1.3	1.3	1.1	0.1	0.1	0.1
Eutric Cambisol	63.5	8.9	0.8	3.7	16.5	2.3	0.2	2.3	0.8	0.2	0.3
Vertisol	70.3	8.8	1.4	2.5	10.8	4.0	0.3	1.1	0.2	0.0	0.3
Distric Cambisol + Luvisol	69.1	7.4	0.8	4.7	13.5	2.3	0.3	1.3	0.3	0.0	0.4
Regosol + Ranker	58.9	6.9	0.8	4.7	18.9	2.5	0.4	1.9	2.5	2.1	0.4
All soils	57.6	8.8	0.7	5.0	18.7	2.0	0.3	1.6	2.5	2.6	0.2

The results of the X-ray diffraction analysis of the clay fraction are presented in Table 2. It has revealed abundant illite associated with smectites and mixed layer silicates (MSS) of the illite/smectite (10—14) and chlorite/vermiculite (14—14) types. They are accompanied by minor amounts of vermiculite, kaolinite, chlorite, quartz, feldspars and rare calcite, dolomite and goethite. A small amount of talc was detected only in several specimens from luvisols and rankers developed on Paleozoic schists in Šumadija. The persistence of talc in fine clay throughout some luvisol profiles could also suggest a high stability of this mineral within soils in strongly leaching and weathering conditions.

Illite as a weathering product of micas is following their spatial distribution. It is more abundant in less weathered soils such as chernozem and semigleys, while it is up to twice less abundant in distric cambisols and luvisols, where strong weathering had occurred during pedogenesis. On the other side smectites could be inherited from parent rocks as in vertisols formed on lacustrine sediments in Šumadija or geochemically developed in highly moist, temporarily flooded or water logged soils such as semi-, humo- and eugleys and fluvisols in Vojvodina. Vermiculite occurs as an illite and/or chlorite transformation product in soils with slightly acid weathering conditions such as distric cambisols, luvisols and pseudogleys. Interstratified minerals of the (10—14) and (14—14) types are abundant in the soils (halomorphic soils and pseudogleys) where particular weathering occured, indicating initial stages of illite and chlorite transformations to either smectite or vermiculite. Kaolinite occurs either as inherited as an alteration product of feldspar in rankers and regosols or it occurs as direct weathering product of feldspars in the soils with strong leaching conditions such as distric cambisols and luvisols.

Agrochemical results

The results of 1M ammonium acetate soluble magnesium extracted from the soils (A horizon) and within soils are presented in Table 3 and Graph 1. Spatial distribution of available magnesium content over the investigated area is presented in agrochemical map in Figure 1.

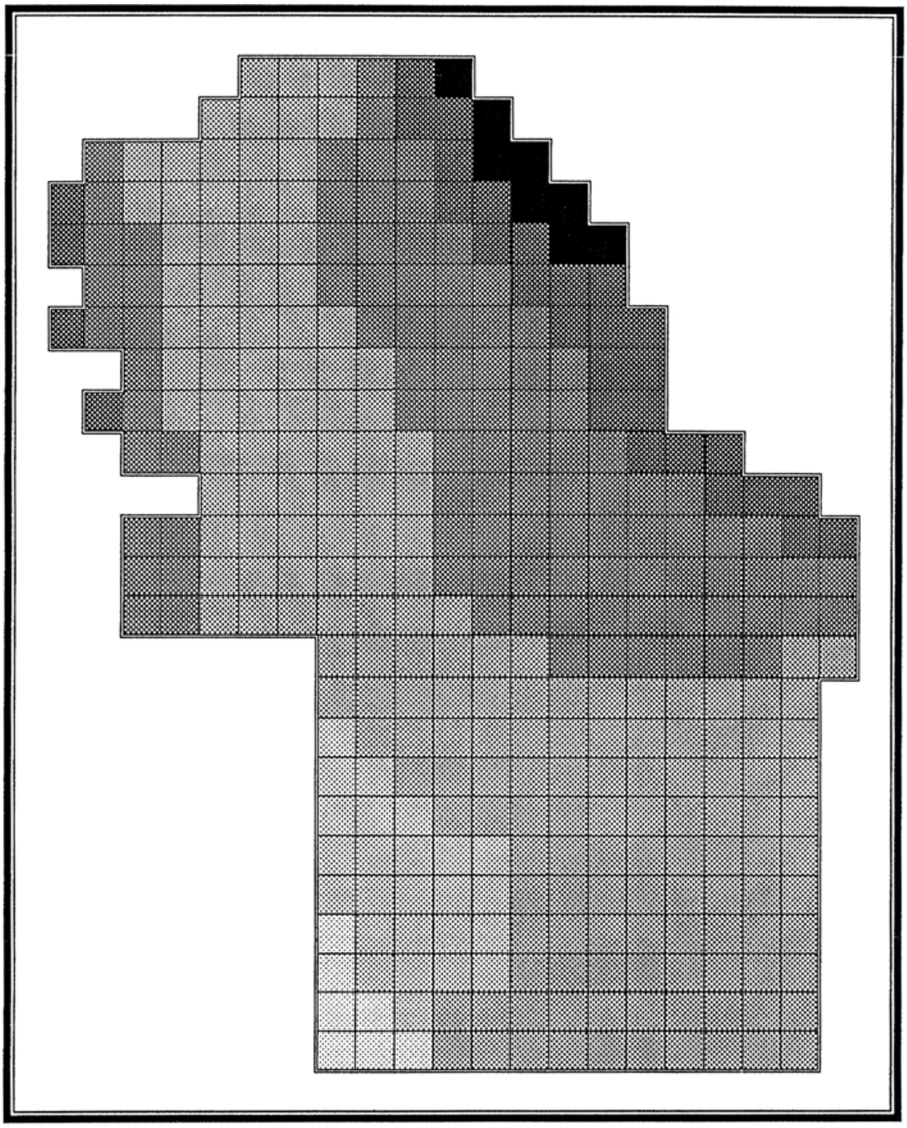
This investigation has shown that the investigated oils have available magnesium content between 10 and 93 mg/100 g, with an average of 40.8 mg/100 g. All investigated soils are well-supplied with available magnesium and could be considered as magnesium sufficient for plant production. It appears that available magnesium is primarily associated with carbonate fraction (dolomite) and to a lesser extent with exchangeable (clay minerals) and silicate forms (mica, chlorite, amphibole).

Two areas can be generally distinguished on the agroecological map. In the first, soils are formed on recent loess and alluvial sediments, as in Vojvodina and Pomoravlje. In the second, soils are developed on Mesozoic and Neogene sediments consisting of mudstones, sandstones, flisch and limestones that run through most of Šumadija.

Tab. 3 — Statistical summary for available magnesium (mg/100 g) classified according to soil type

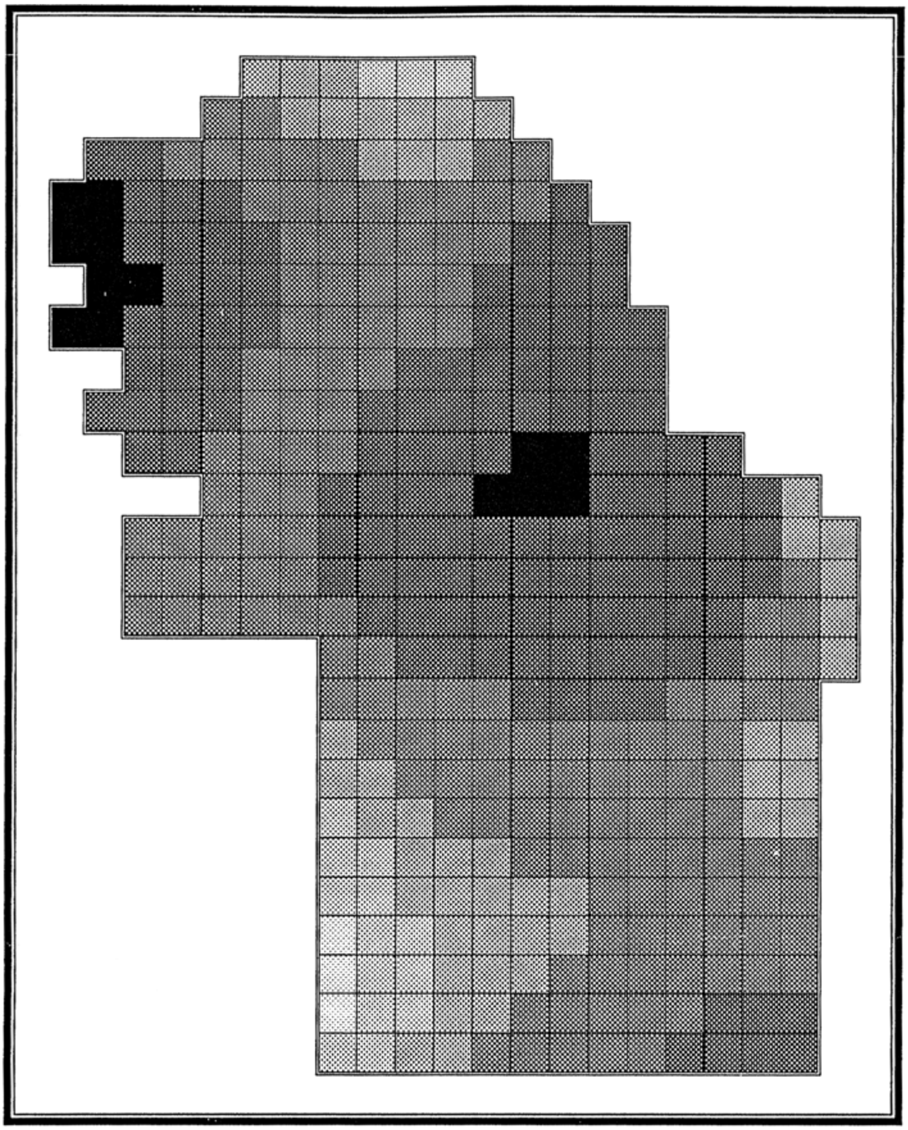
Soil types	Number of samples	Interval		Average Mg	St. dev.
		min	max		
Ranker, regosol and rendzina	6	13	38	22.8	8.83
Distric cambisol and luvisol	5	23	40	32.4	8.46
Vertisol	5	15	38	26.8	9.36
Cambisol	14	23	70	37.3	12.96
Halomorphic soils	5	25	53	36.8	11.19
Pseudogley	5	10	45	32.8	13.74
Chernozem	20	28	65	41.7	10.00
Semigley	20	20	75	43.4	14.00
Fluvisol	8	22	80	48.0	20.90
Eugley and humogley	12	23	93	57.8	20.62
All analyzed soils	100	10	93	40.81	16.12

Initial soils (rankers and regosols), soils developed on Neogene sediments such as vertisols, some eutric and distric cambisols and pseudogleys contain less than the average amount of available magnesium (10—40 mg/100 g). On the other side, soils formed on loess and alluvial sediments (chernozem, semi-



Available magnesium (mg/100g)	< 20	20 - 30	30 - 40	40 - 65	65 - 80	> 80
Raster label	░	░	▒	▒	▓	█

Fig. 1 — Agrochemical map of available magnesium



Total magnesium (%)	<0.2	0.20 - 0.30	0.30 - 0.40	0.40 - 0.65	0.65 - 0.80	>0.8
Raster label	░	░	▒	▒	▓	█

Fig. 2 — Agrochemical map of total magnesium

gley, fluvisol and humo- and eugleys) are comparatively rich in available magnesium (40—93 mg/100 g).

The results of AAS analysis of total magnesium in the soils (A horizon) are presented in Table 4. Spatial distribution of total magnesium content over area and within soils investigated is presented on the geochemical map in Figure 2.

Total content of magnesium varies between 0.20 and 1.26%, with an average of 0.603%. The ratio of available to total magnesium content in soils has indicated that the greater part (80 to 98%) of magnesium was present in residual forms bound to the lithogenic fraction. It appears that total magnesium content is primarily associated with the carbonate fraction (dolomite) as in soils developed on recent alluvial sediments and loess in Vojvodina, to a lesser extent in silicate forms (mica, chlorite, clay minerals, amphibole), so in soils formed on Neogene and Mesozoic sediments that run through most of Šumadija. Exceptions are regosol and rendzina which are formed on parent rocks rich in carbonates.

Tab. 4 — Statistical summary for total magnesium content (%) classified according to soil type

Soil types	Number of samples	Interval		Average (%) Mg	St. dev.
		min	max		
Vertisol	5	0.20	0.52	0.352	0.133
Distric cambisol and luvisol	5	0.32	0.43	0.346	0.052
Pseudogley	5	0.25	0.57	0.422	0.116
Cambisol	14	0.36	0.68	0.476	0.115
Halomorphic soils	5	0.35	0.85	0.568	0.193
Eugley and humogley	12	0.20	1.00	0.614	0.206
Ranker, regosol and rendzina	6	0.39	1.25	0.655	0.333
Chernozem	20	0.25	1.00	0.666	0.176
Fluvisol	8	0.24	1.00	0.740	0.268
Semigley	20	0.26	1.26	0.744	0.264
All analyzed soils	100	0.20	1.26	0.603	0.235

The Ca/Mg ratio of available forms has shown a large scale variation, between 2.5:1 and 78.8:1. Only 10% of the soils have the Ca/Mg ratio lower then 5:1 or optimal ratio for plant nutrition, while 90% of the soils were found to have the ratio above 5:1 which might be a limiting factor for growth of some plants, cause problems in agricultural production and hamper an adequate supply of magnesium to animals and men.

From statistical correlation analyses it is evident that total magnesium content is closely correlated with the mica + illite (r = 0.87) and chlorite (r = 0.74) contents as well as to the carbonate — dolomite (r = 0.72) content of the investigated soils. Available magnesium is also positively correlated with micas + illite (r = 0.70) and chlorite (r = 0.60) but to a lesser extent to smectite + vermiculite (r = 0.33) and dolomite (r = 0.33).

The correlation of total and available magnesium contents with minerals in the clay fraction has shown slight difference from that for bulk soil mineralogy. Total magnesium content has revealed a high positive correlation with fine dolomite particles in the clay fraction (r = 0.72), while available magnesium has shown a slight positive correlation with smectite (r = 0.39) and dolomite (r = 0.38) in all investigated soils.

Analysis of correlation data for specific soils has pointed out semigley as the only soil with a positive correlation of both total and available magnesium with dolomite in the bulk soil, while in the clay fraction they are correlated with clay chlorite. The weathering processes may affect soil mineralogy in the soils as well as soil mineral correlations with total and available magnesium contents. Fluvisols, chernozem and eutric cambisols have shown positive correlations among magnesium and micas + illite and chlorite in the bulk soils, while in the clay fractions magnesium correlated with interstratified minerals, smectite and vermiculite. Magnesium was correlated with smectite in both bulk and clay fractions in luvisols, distric cambisols and pseudogleys. Bulk samples of vertisols, humo- and eugleys have shown magnesium correlations with chlorite, amphiboles and interstratified minerals of (14—14) type, while in clay fraction magnesium had positive correlations with illite and interstratified illite/smectite.

CONCLUSIONS

Magnesium is an important element of the biosphere, hydrosphere and lithosphere. It makes up to 2.5% by weight of the Earth's crust and is invariably present in all soils. The greatest part of magnesium occurs in the crystal lattices of numerous minerals, which are the main source of magnesium in nature.

In humans biosphere, magnesium is an essential macronutrient for plants, animals and humans. Magnesium deficiency in humans could be linked to the pathogenesis of cancer, or might be related to sudden cardiac deaths in areas with soft water (McMillan, 1978).

This paper is concerned with the magnesium content in the most important soil types of Vojvodina, Šumadija and Northern Pomoravlje.

The available magnesium in the investigated soils is primarily associated with the carbonate fraction e.g. mineral dolomite ($CaCO_3$ x $MgCO_3$), and to a lesser extent with the exchangeable (clay minerals) and silicate forms (mica, chlorite, amphibole). The greatest part of magnesium (80 to 98%) was present in residual forms bound to the lithogenic fraction.

This investigation has shown that the soils in Serbra contain between 0.20 up to 1.26% Mg, with an average content of_0.603%. The available magnesium content varies between 10 and 93 mg/100 g, with an average content of 40.8 mg/100 g Mg.

Taking in account both total and available magnesium contents it could be concluded that the investigated soil contain enough magnesium for proper plant nutrition. However, magnesium availability is largely determined by the ratios between available cations Ca and K with Mg.

The Ca/Mg ratio of available forms has shown a large scale variation, between 2.5:1 and 78.8:1. Only 10% of the soils have the Ca/Mg ratio lower then 5:1 which is optimal for plant nutrition. The K/Mg ratio of available forms is on average 1.1:1, and would not affect magnesium uptake, while the ratio higher than 4:1 could be considered as a limiting factor for available magnesium uptake and utilization by plants.

About 90% of the soils were found to have the ratio above 5:1 which might be a limiting factor for growth of some plants. This could become a problem in the case of a wide Ca/Mg ratio and when available magnesium content is near the limit of deficit (< 10 mg/100 g), as well as in the case of plants sensitive to available magnesium content in soils.

This paper has shown a need for a wider investigation of the chemistry and mineralogy of soils in Serbia, which are generally considered as well supplied with magnesium. However, deficit of available magnesium in soils combined with high ratios of Ca/Mg and K/Mg could become a limiting factor in plant physiology, particularly to the plants with high demand for magnesium and favorable ratios of available cations such as Ca, K, Na, H and Mg.

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МИНЕРАЛОГИЈА И АГРОХЕМИЈА МАГНЕЗИЈУМА У ЗЕМЉИШТИМА ВОЈВОДИНЕ, ШУМАДИЈЕ И СЕВЕРНОГ ПОМОРАВЉА

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Резиме

У раду су анализирани садржаји и дистрибуција укупног и приступачног магнезијума у земљиштима Војводине, Шумадије и северног Поморавља.

Магнезијум је врло важан елемент у грађи биосфере, хидросфере и скоро свих стена литосфере, где изграђује многобројне минерале, који су основни извор магнезијума у природи. У биосфери магнезијум је неопходан макрохранљиви елеменат за биљке, животиње и људе. Недостатку магнезијума у исхрани људи посвећује се све већа пажња, јер може да се повеже са патологијом рака и болести срца у подручијима са меком водом McMillan (1978).

Основни извор приступачног магнезијума у нашим земљиштима је првенствено минерал доломит, а у мањој мери минерали глина и други алумосиликати: хлорити, лискуни и амфиболи. Највећи део (80—90%) магнезијума у испитиваним земљиштима је везан за литогену — силикатну фракцију.

Ово истраживање је показало да земљишта у Србији садрже укупни магнезијум од 0.20 до 1.26% са просеком од 0.603%, док садржај приступачног магнезијума варира између 10 и 93 mg/100 g са средњим садржајем од 40.8 mg/100 g. С обзиром на садржаје укупног и приступачног магнезијума, може се сматрати да су наша земљишта сасвим довољно обезбеђена овим елементом, за правилну исхрану биљака. Међутим, велику важност за приступачност магнезијума имају и односи приступачних катјона калцијума, калијума и магнезијума.

Однос приступачног Ca/Mg варира у широким границама између 2.5:1 и 78.8:1. Међутим. само 10% испитиваних земљишта има Ca/Mg однос нижи од 5:1 т. ј. оптималан однос за исхрану биљака. Однос K/Mg је просечно износио 1,1:1, што указује да овај однос неће негативно утицати на усвајање Mg. Само односи већи од 4:1 уз дефицит приступачног магнезијума сматрају се негативнима за усвајање Mg.

Око 90% земљишта има повишен однос изнад 5:1, што може бити ограничавајући чинилац за раст биљака и проузроковати неке проблеме у пољопривредној производњи и тако утицати на снабдевање људи и животиња неопходним магнезијумом. Ови проблеми настају при сувише широким односима (Ca/Mg) и када је садржај приступачног Mg близу границе дефицитарности (< 10 mg/100 g Mg).

Ово истраживање указало је на потребу ширег проучавања хемије и минералогије наших земљишта, за која је досада сматрано да су добро снабдевана магнезијумом, јер у мањем броју случајева може доћи до поремећаја у усвајању Mg (при мањој приступачности Mg, уз повећање односа Ca/Mg и K/Mg).

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THE ROLE OF PROTON EXCHANGEABLE CALCIUM IN THE HOMEOSTASIS OF INTRACELULLAR CALCIUM IN PREFUSED AND LIGATED INTERNODAL CELLS OF *Chara gymnophylla*

ABSTRACT: It is known that calcium ions present in the cell wall have some important functions related to the structural and mechanical properties of the cell wall, cell growth, the control of ionic diffusion across the cell wall, the function of membrane transporters and their own membrane fluxes. However, it seems that so far nobody has investigated the influence of cell wall calcium on its intracellular homeostasis. Internally perfused and ligated internodal cells of a freshwater alga, *Chara gymnophylla*, loaded with fura-2 were used to study the effects of removal of proton exchangeable calcium from the cell wall (by „decalcification") on the intracellular Ca^{2+} homeostasis. Decalcification of the cell wall induced an increase of resting Ca^{2+} level, probably indirectly by reducing the activity of plasmalemmal proton pump. The decalcification also lowered the relative amplitude of D_2O-induced Ca^{2+} rise by diminishing the intracellular Ca^{2+} concentration gradient across the cell membrane. Beside that, the decalcification reduced the rate of D_2O-induced influx as well as the efflux (in a Ca^{2+}-free external solution) of calcium. This can be explained by electrostatic interaction of the cell wall calcium with plasmalemmal calcium channels, which influences the channels in such a way that the lowering of calcium concentration in the cell wall reduces its activity.

KEY WORDS: *Chara*, calcium, decalcification, cell wall

INTRODUCTION

The role of calcium in intracellular signal transduction in Characean internodal cells is discussed for many phenomena such as action potentials (Lunevsky et al., 1983; Pliethet al., 1998), action of D_2O (Andjus et al., 1997, Zivic and Andjus, 2001), cytoplasmatic streaming (Williamson and Ashley, 1981), wounds healing (Foissner, 1998) and effects of light (Plieth et al., 1998). The main source of calcium for the internodal cells is extracellular solution. But, in contrast to animal cells whose plasmalemma is in direct contact with extracellular environment, the plasmalemma of characean internodal cells, just like in all plant cells, communicates with extracellular solution via cell wall. The cell wall of *Charophyta* acts as a porous leaky cation exchanger (Dainty and Hope, 1959; Gillet and Lefebvre, 1978; Greenleaf et al., 1980), and therefore the ions crossing a cell wall interacts with its negative fixed charges before they penetrate the plasmalemma. Because of these interactions, cations are selectively absorbed by the cell wall and their activity coefficients are altered, which in turn may affect the transmembrane potential and the electrolyte (e.g. calcium) flow across the plasmalemma (Van Custem and Gillet, 1982).

Calcium in the cell wall is found to be loosely held in a diffuse double layer together with other cations and chelated by acid-labile wall bonds. The chelated calcium, in contrast to that in the diffuse double layer, is not exchangeable with monovalent ions even at large concentrations and can be removed from the cell wall only by the removal of calcium from the medium or by acidification, so it is known as proton exchangeable calcium (Gillet and Lefebvre, 1980).

It is shown that calcium ions present in the cell wall have several functions: they are, to a large extent, responsible for its structural and mechanical properties and in this way related to cell growth (Probine and Preston, 1962; Arens et al., 1972); by influencing the diffusion coefficients, they participate in the control of ionic diffusion across the cell wall (Gillet and Lefebvre, 1978); calcium exerts influence on the function of membrane transporters (Leonard et al., 1975), and its own membrane fluxes (MacRobbie and Banfield, 1988) by altering the electrostatic field of the fixed charges. However, nobody so far seems to have investigated the influence of cell wall calcium on its intracellular homeostasis.

The aim of this paper is to examine the influence of removal of proton exchangeable calcium from the cell wall of characean internodal cells on the homeostasis of calcium in resting state as well as on the course of change of calcium concentration induced by the application of heavy water in the external medium.

MATERIALS AND METHODS

Measurements were performed on the giant internodal cells (1—2 cm long) of the freshwater alga *Chara gymnophylla* grown in the laboratory. For the purpose of controlling the cytosolic environment and loading the fura-2 dye, a modified preparation of a perfused and ligated internodal cell introduced by Tazawa (Tazawa, 1961) was used. Prior to the experiment, isolated internodal cells were internally perfused with calcium buffer *Ca-50* (in mM: KCl 150, EGTA 0.9, $CaCl_2$ 1.06, citrate 0.5, sucrose 100, Hepes-KOH 20, pH 7.2) containing 50 μM free $Ca^{2+}$and 100 μM. calcium-sensitive fluorescence probe fura-2 (Sigma). The internodal cells were then ligated at both ends and fixed in the experimental chamber.

Experiments were performed in external calcium buffer (in mM: KCl 150, EGTA 0.9, $CaCl_2$ 1.154, citrate 0.5, sucrose 100, Hepes-KOH 20, pH 7.2) with 150 μM free Ca^{2+}.

In order to investigate the influence of Ca^{2+} from the free spaces of the cell wall, a set of experiments was performed on cells which were kept in stirred Ca^{2+}-free APW (in mM: KCl 0.4, NaCl 1,2-N-morpholino ethanesulfonic acid, 5; pH 5) for 2 h with solution changes every 30 min (MacRobbie and Banfield, 1988). These „decalcified" cells were left overnight in APW, before the experiment.

The solutions were applied by means of gravity. Calcium buffers in D_2O were used during D_2O/H_2O exchanges. The D_2O solution was made so that its pD (pD ≈ measured pH + 0.4, Glasoe and Long 1960) was equal to the pH of the pre-treatment H_2O solution. D_2O utilized in these experiments was 99.8% pure (Fluka).

The experimental chamber was placed on the stage of an inverted microscope (Axiovert 10; Opton, Germany) supplied with a xenon lamp light source and equipped for epifluorescence. The excitation light of 360 and 390 nm was alternated (2 Hz) by means of a motorized filter wheel and deflected to the sample through a 20x Zeiss-plan Neufluar objective. The emitted fura-2 fluorescence was transmitted to a photomultiplier unit (Luigs & Neumann, Germany). The intensity of fluorescence emission was adjusted to subsaturating levels by the use of a diaphragm and a neutral density filter (diminishing light intensity down to 12%) in the light path. With fluorescence values corrected for background and dark currents, calculation of $[Ca^{2+}]_i$ was carried out on an Atari ST Mega computer from the ratio (R) between 360 and 390 nm recordings, according to the equation: $[Ca^{2+}]_i = K_d \times [(R — R_{min}) / (R_{max} — R)] \times \beta$ (Grykiewicz et al., 1985). Based on *in situ* calibration (Thomas and Delaville, 1991) on decalcified cells, the parameters $R_{min} = 3.43$, $R_{max} = 144.31$, and $\beta = 39.53$, with the effective K_d, $K_{eff} = K_d \times \beta = 8854$ nM, were specified in the calibration menu. Unless otherwise stated, the data were presented as relative calcium concentration ($[Ca^{2+}]_{rel}$), i.e. as percentage of basal (resting) $[Ca^{2+}]_i$, and expressed as mean ± S.E.M.

The samples were compared statistically using Mann-Whitney U-test at the 5% level of significance ($P < 0.05$).

RESULTS

The *in situ* calibration (Fig. 1) on the cells with intact cell wall was unsuccessful because the adding of the Ca ionophore (4-BrA23187) in the presence of a sharp electrochemical gradient of calcium directed toward the external solution provoked only a small drop in fluorescence ratio (from R = 60 to R = 50). This could not be attributed to the malfunction of the ionophore because a large increase of fluorescence ratio occurred when calcium was added in excess (from 50 to 150, 50 m; $CaCl_2$ added). The only plausible explanation is that the exchangeable calcium in the cell wall significantly lowers its transmembrane electrochemical gradient. This presumption was confirmed by the *in situ* calibration on the cells with decalcified cell wall. In this case, the adding of ionophore provoked a large drop in fluorescence ratio (from R = 50 to R = 3.43) while the rise stayed the same. This was a clue for performing addi-

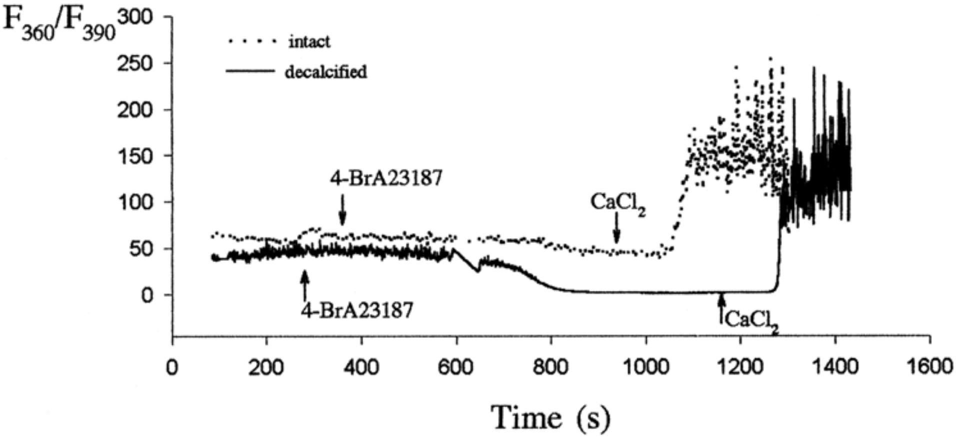

Fig. 1 — Changes of fluorescence ratios during the in situ calibrations of cell preparations with intact (solid line) and decalcified (dashed line) cell walls. Arrows marks the adding, in the calcium free external solution (in mM: KCl 150, EGTA 4, Tris 30 and sucrose 100, pH = 5.0), of 10 μM calcium ionophore 4-BrA23187 („4-BrA23187"), and 50 mM $CaCl_2$ („$CaCl_2$").

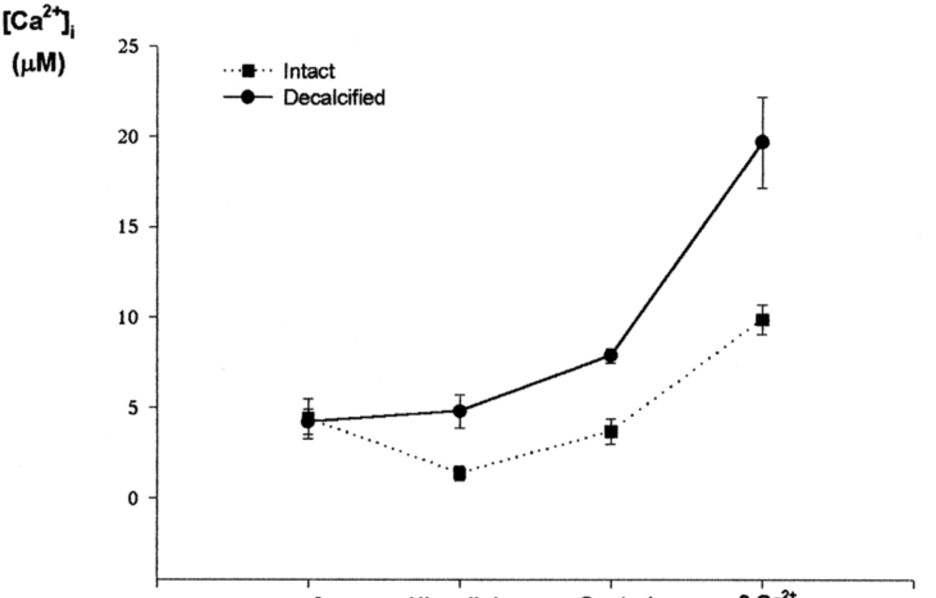

Fig. 2 — Resting $[Ca^{2+}]_i$ in cell preparations with intact (dashed line and squares) and decalcified (solid line and circles) cell walls. Results are given for external solution containing 150 μM free Ca^{2+} („control") and no free Ca^{2+} („0Ca^{2+}"), and for cells pretreated with 0.5 mM $LaCl_3$ („La^{3+}"), inhibitor of cationic transmembrane transport, and 50 μM nicardipine („nicardipine"), inhibitor of Ca^{2+} channels, with 150 μM free Ca^{2+} in the extracellular solution in last two cases.

tional experiments concerning the influence of calcium in the cell wall on the calcium homeostasis in the cell.

In the first set of experiments, attention was focused on the influence of decalcification of the cell wall on the resting calcium level. Resting calcium concentration in the cells with the intact cell wall (3.7 ± 0.7 μM, n = 12) was significantly lower ($p < 0.001$) compared with that in the cells with decalcified cell wall (7.9 ± 0.4 μM, n = 21; Figure 2). The resting $[Ca^{2+}]_i$ increased even further in Ca^{2+} free extracellular solution (no added Ca^{2+}, in the presence of 0.9 mM EGTA), both in cells with intact cell wall (9.9 ± 0.8 μM, n = 8) and those with decalcified cell wall (19.7 ± 2.5 μM, n = 6; Figure 2), maintaining the significantly higher free calcium content in decalcified cells ($p < 0.05$). In order to obtain information about the cause of this difference, the cells were pretreated with nicardipine (blocator of calcium channels) and lanthanum (blocator of cationic channels). In cells pre-treated with nicardipine there was a significant decrease of basal $[Ca^{2+}]_i$ as compared with untreated cells, namely to 1.4 ± 0.4 μM (n = 5) and to 4.8 ± 0.9 μM (n = 6) in intact and decalcified cells, respectively (Figure 2). Again a significantly ($p < 0.05$) higher free Ca^{2+} content in decalcified cells was found. However, the difference between the intact and decalcified cells is eliminated ($p > 0.05$) with La^{3+} pre-treatment (Figure 2), $[Ca^{2+}]_i$ being 4.4 ± 1.1 μM (n = 6) and 4.2 ± 0.7 μM (n = 7), respectively. The former value did not significantly differ from the control, however, the latter resting $[Ca^{2+}]_i$ value in decalcified cells was significantly lower as compared with the decalcified control (*see above*, $p < 0.01$).

In the second group of experiments, attention was focused on the effects of decalcification of the cell wall on the change of calcium concentration induced by D_2O.

The main effect of D_2O application on the perfused and ligated internodal cell of *C. gymnophylla* was an increase in $[Ca^{2+}]_i$ preceded by a transient $[Ca^{2+}]_i$ decay (Figure 3 A, B). Internodal cells could be separated into two types — L (large response) or S (small response) type. The L-type cells were characterized by a lower amplitude of transient $[Ca^{2+}]_i$ decay and a larger and slower $[Ca^{2+}]_i$ increase as compared with the S-type cells (Živić and Andjus, 2001).

In the L-type cells with intact cell wall, the amplitude of increase was 42 ± 3% (n = 7), the amplitude of decay –9 ± 2% (n = 6), while the time constant of increase was 12 ± 1 s (n = 7). The decalcification of cell wall induced a smaller (30 ± 2%, n = 9) and slower (τ = 21 ± 2 s, n = 9) increase in response to D_2O application as compared with the cells with intact cell wall, while the amplitude of transient decrease (–10 ± 1%, n = 9) was not significantly different. However, in the case of the S-type cells with intact and decalcified cell walls there were no significant differences regarding any parameter of D_2O response: the amplitudes of transient decay were –22 ± 3% (n = 5) and –16 ± 1% (n = 12), the amplitudes of increase 14 ± 1% (n = 5) and 15 ± 1% (n = 12) and the time constants 8 ± 1 s (n = 5) and 8 ± 1 s (n = 12), respectively.

In order to investigate the influence of removal of calcium from the cell wall on the efflux of calcium, D_2O treatment of cells with intact and decalcified cell walls was performed in the external solution with no free calcium.

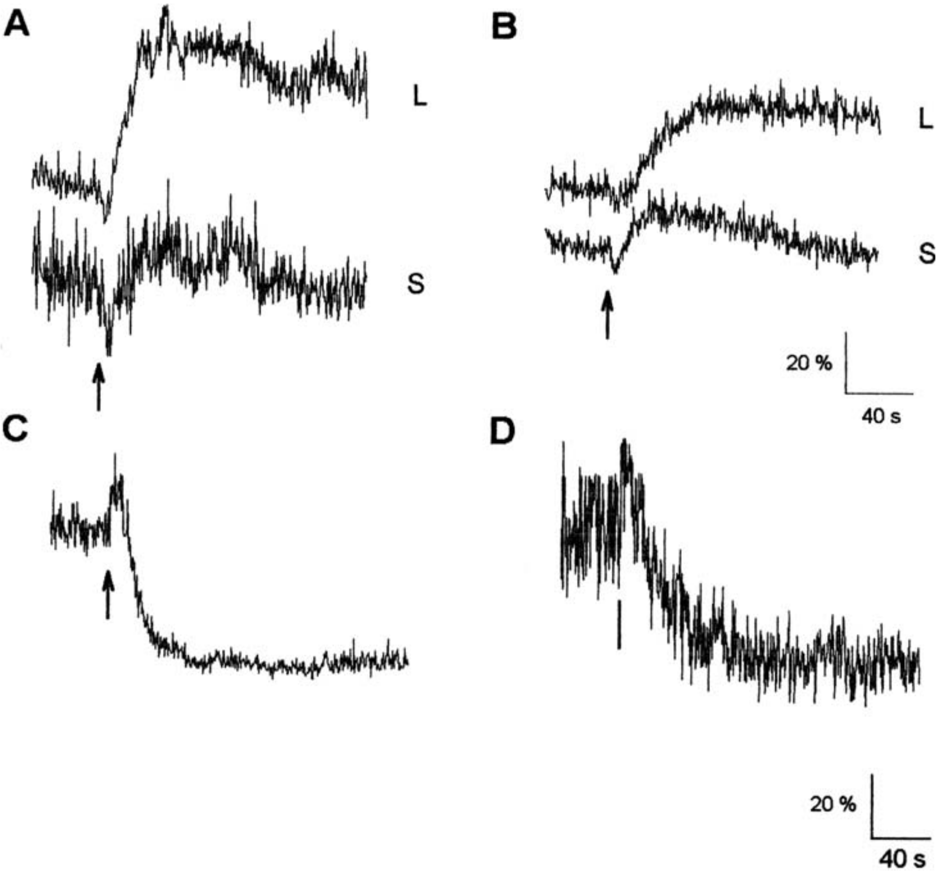

Fig. 3 — Average traces of $[Ca^{2+}]_{rel}$ responses to D_2O application (at arrows) of cells with intact (A and C) or decalcified (B and D) cell walls in external solutions containing 150 μM free Ca^{2+} (A and B) or no free Ca^{2+} (C and D). With „S" and „L" are labeled average responses to D_2O of the L- and S-type (see text).

Thus, in a Ca^{2+}-free medium, D_2O first induced a transient (≈10 s) increase in $[Ca^{2+}]_i$, followed by a prominent decrease to a plateau level (Figure 3 C, D). The responses to D_2O in the cells with intact and decalcified cell walls showed no statistically significant differences in $[Ca^{2+}]_{rel}$ changes (15 ± 3 % vs. 21 ± 3 % for the maximal rise and –48 ± 4 % vs. –51 ± 4% for the maximal decrease, respectively). The rate of Ca^{2+} decrease was significantly higher in intact cell preparations as compared with decalcified ones (τ was 12 ± 1 s as compared with 48 ± 8 s, respectively).

DISCUSSION

The results of this study showed that the removal of proton exchangeable calcium from the cell wall of characean internodal cells had significant effects on both, the homeostasis of calcium in the resting state and the change of calcium concentration induced by the application of D_2O.

In the resting state, decalcification induced a significant increase of calcium concentration. Only La^{3+} eliminated the difference in resting $[Ca^{2+}]_i$ between cells with intact and decalcified cell walls, probably by substituting calcium in the free spaces of the cell wall (Ried and Smith, 1992). Since the removal of calcium from extracellular solution (Figure 2) increases resting $[Ca^{2+}]_i$ too, it can be concluded that decalcification acts on the resting $[Ca^{2+}]_i$ by lowering calcium concentration in the vicinity of the plasmalemma. The mechanism by which the lowering of external calcium concentration acts on the resting $[Ca^{2+}]_i$ is not clear but it could be explained by a model based on the fact that the lowering of external calcium concentration reduces the activity of proton pump in the plasmalemma of characean internodal cells (Bisson, 1984). It was known, in the case of several higher plants, that the reduction of proton pump activity and subsequent acidification of the cytoplasm led to the increase of $[Ca^{2+}]_i$ (Cervantes — Cervantes and Bush, 1994). The link between the acidification and increase of $[Ca^{2+}]_i$ could be plasmalemmal Ca^{2+}/nH^+ antiporter, which uses H^+ gradient across the plasmalemma for active efflux of calcium. So, the disruption of the H^+ gradient reduces the activity of the antiporter and subsequently increases $[Ca^{2+}]_i$. In our cell preparations where $[Ca^{2+}]_i$ was higher than in intact cells ($[Ca^{2+}]_i$ in the perfusion media is 50 μM), the above mechanism of increase of resting $[Ca^{2+}]_i$ upon decalcification was even more probable due to the lower activity (Bush, 1995) and higher maximal velocity of calcium transport by the antiporter compared with the second ubiquitous active Ca^{2+} transporter, Ca^{2+} ATPases.

Decalcification of the cell wall induced the lowering of relative amplitude, $[Ca^{2+}]_{rel}$, and slowing of $[Ca^{2+}]_i$ rise upon D_2O application, but only in the L-type preparations. Taking into consideration that the S-type responses occur in consequence to calcium release from the internal stores, while the L-type responses are consequences of both, calcium release from internal stores and its efflux from extracellular solution (Živić and Andjus, 2001), it can be concluded that decalcification of the cell wall modifies the parameters of calcium response to D_2O through the action on plasmalemmal calcium transporters. The slowing of $[Ca^{2+}]_{rel}$ increase is a consequence of diminished calcium concentration gradient upon decalcification, which is congruent with previous findings that exchangeable calcium from the cell wall is an active component of extracellular calcium in a whole array of processes (see *in situ* calibration, Figure 1, and Bush, 1995). The lower rates of D_2O-induced Ca^{2+} influx (Figure 3 A, B) and efflux (0 Ca^{2+} in the extracellular media, Figure 3 C, D) upon decalcification can be explained by electrostatic interaction of the cell wall calcium with membrane proteins (Grignon and Sentenac, 1991), in our case with calcium channels. This interaction with calcium channels most probably influences the activity of the channels in such a way that the lowering of calcium concentration in the cell wall reduces their activity.

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УЛОГА ПРОТОН ИЗМЕЊИВОГ КАЛЦИЈУМА ИЗ ЋЕЛИЈСКОГ ЗИДА У ХОМЕОСТАЗИ УНУТАРЋЕЛИЈСКОГ КАЛЦИЈУМА КОД ПЕРФУНДОВАНЕ И ПОДВЕЗАНЕ ИНТЕРНОДИЈАЛНЕ ЋЕЛИЈЕ АЛГЕ *Chara gymnophylla*

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Резиме

Познато је да јони калцијума присутни у ћелијском зиду имају веома важне функције везане за структурне и механичке особине ћелијског зида, ћелијски раст, контролу јонске дифузије кроз ћелијски зид, функцију мембранских транспортера и сопствене мембранске флуксеве. Међутим, нико досада није испитивао утицај калцијума из ћелијског зида на хомеостазу унутарћелијског калцијума. Перфундоване и подвезане интернодијалне ћелије слатководне алге *Chara gymnophylla* напуњене са фура 2 коришћене су за проучавање ефеката уклањања са протонима измењивог калцијума из ћелијског зида (помоћу „декалцификације”) на хомеостазу унутарћелијског калцијума. Декалцификација ћелијског зида је изазвала пораст мирујућег нивоа калцијума, вероватно посредно, преко редукције активности протонске пумпе на ћелијској мембрани. Декалцификација је такође довела до смањивања релативне амплитуде D_2O изазваног пораста концентрације унутарћелијског калцијума смањујући концентрациони градијент калцијума преко ћелијске мембране. Поред тога декалцификација је изазвала опадање брзине D_2O изазваног уласка као и изласка (у спољашњем раствору без калцијума) калцијума. Ово може бити објашњено електростатичким интеракцијама калцијума из ћелијског зида са калцијумовим каналима у ћелијској мембрани, које на канале делују на такав начин да смањење концентрације калцијума у ћелијском зиду доводи до смањења њихове активности.

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EFFECTS OF CHANGES IN THYLAKOID MEMBRANES — A MEASURE FOR EVALUATION OF RESISTANCE AND ADAPTABILITY OF MAIZE INBRED LINES TO HIGH TEMPERATURE

ABSTRACT: The chlorophyll delayed fluorescence (DF) method, as a non-invasive bioluminescence method, is recommended for application in maize breeding and seed production in order to evaluate maize inbred lines for resistance and adaptability to increased and high temperatures.

The following thermal properties of the photosynthetic apparatus of the studied maize inbred lines have been determined: the temperature dependence within a range of 23—60°C; critical temperatures at which phase transitions occur in the thylakoid membrane were established and by means of it significant functional changes in the photosynthetic apparatus of the investigated maize inbred lines were detected. Activation energies (E_a, kJ mol^{-1}) alongside the straight lines prior and after critical temperatures were calculated. E_a is a measure of occurrence of chlorophyll DF recombination processes and, simultaneously, a measure of total changes in the structure and functioning of thylakoid membranes.

Thylakoid membranes in maize inbred lines underwent significant conformational changes in the part following the maximum intensity, i.e. in the part of its sharp linear decline. Such a state fits more a non-living than a living organism in relation to its both, structure and functions.

The presented parameters of total thermal processes of chlorophyll DF such as temperature dependence, critical temperatures and activation energy can be an important factor for a more exact characterization of maize inbred lines in relation to their resistance and adaptation to increased and high temperatures, contributing to a more rapid and rational development of the selection process.

KEY WORDS: maize inbred lines, thylakoid membrane, photosynthetic process, delayed fluorescence, thermal process, critical temperature, activation energy

INTRODUCTION

Although photosynthetic processes are ubiquitous, highly intensive, very complex and broadly observed, they still do not have sufficient application in plant breeding. This situation is probably a consequence of the existence of several functional interrelationships that unite structural and dynamic changes within chloroplasts and their thylakoid membranes on the one hand, and effects of numerous environmental factors on the other.

Chlorophyll DF can be phenomenologically described as the occurrence of luminescence (bioluminescence) within the red range of the visible spectrum produced by plant systems, bacteria, algae and higher plants, immediately after their intermittent illumination (Radenović, 1992, 1994, 1997). Chlorophyll DF was discovered by Stehler and Arnold (1951) in their attempt to reveal a nature of induction illumination in a form of bioluminescence. Numerous studies, especially those conducted over last 20 years (Jursinic 1986, Marković et al., 1993, 1996, Veselovski and Veselova 1990), revealed a direct connection between chlorophyll DF and photosynthetic processes, in which DF was considered an unavoidable indicator — a susceptible „probe" for experimental photosynthetic studies (Radenović et al, 1994a, 1994b, Radenović and Jeremić, 1996, Marković et al., 1987, 1993, 1996, 1999). Chlorophyll DF has become, and it will remain in immediate future, a modern methodological approach in the study of certain photo-processes in the light phase of photosynthesis. Consequently, thermal processes of chlorophyll DF and the application of activation energy in these processes are topical issues (Radenović, 1997, Radenović and Jeremić, 1996).

During last 20 years, attempts were made at the Maize Research Institute in Zemun Polje to employ complex photosynthetic processes in characterizing maize inbred lines. Such an approach can improve the cost efficiency of the breeding process. Therefore, a new, non-invasive method for chlorophyll DF measurements was developed. The measurements were performed on intact leaves of maize inbred lines (Radenović, 1997, Vučinić et al., 1982, Marković et al., 1987, 1993, 1996) and introduced into maize breeding.

The objective of the present study was to determine effects of increased and high temperatures on changes of thermal processes of chlorophyll DF in thylakoid membranes of observed maize inbred lines. The obtained changes in chlorophyll DF intensities, activation energies, as well as the different values of critical temperatures at which evident conformational changes in thylakoid membranes occurred can be good parameters for the evaluation of resistance and adaptation of the observed maize inbred lines to increased and high temperatures.

MATERIAL AND METHODS

Maize inbred lines from the collection of the Maize Research Institute, Zemun Polje, were used as objects in this study. Two inbreds (ZPPL 14 and ZPPL52) were developed at the Institute, while the inbred H108 is of the USA origin.

The principal traits of these inbreds are:

1) The inbred ZPPL 14, derived from crosses of the inbred A82 with a multirow dent population originating from Serbia, belongs to the FAO maturity group 450. Its dent-type kernel is yellow, the cob is red.

2) The inbred H108 of the FAO maturity group 550 is derived from the cross (Mo17 x H98) x Mo17, and it belongs to USA public inbreds. Its dent-type kernel is yellow, the cob is red.

3) The inbred ZPPL 52, derived from self-pollinated maize varieties originating from Istria, belongs to the FAO maturity group 600. Its flint — semi-flint kernel is red-yellow in color, the cob is white. This inbred is a component of commercial ZP maize hybrids.

Figure 1 shows a schematic presentation of the measuring equipment for chlorophyll DF. This scheme of the bioluminescence method has been developed and mainly used at the Biophysical Laboratory of the Maize Research Institute, Zemun Polje. Measurements of changes in chlorophyll DF intensities were performed after a method that was both, in principle and detail, described in previous papers (Radenović, 1979, 1992, 1994, 1997; Marković et al., 1996).

The plant materials were grown at the experiment field of the Institute. During July and August, maize plants were taken to the laboratory during morning hours (between 7 am and 8 am). Plants, when sampled in the field, were transversely cut at the ground internode. In the laboratory, plants were internode lengthwise placed in water. Two hours prior to the bioluminescence experiment, plants were kept under the ball glass. A segment of intact leaf was taken from such plants and placed into the chamber of the phosphoroscope and kept (in dark) for at least 15 minutes, and then measured for chlorophyll DF.

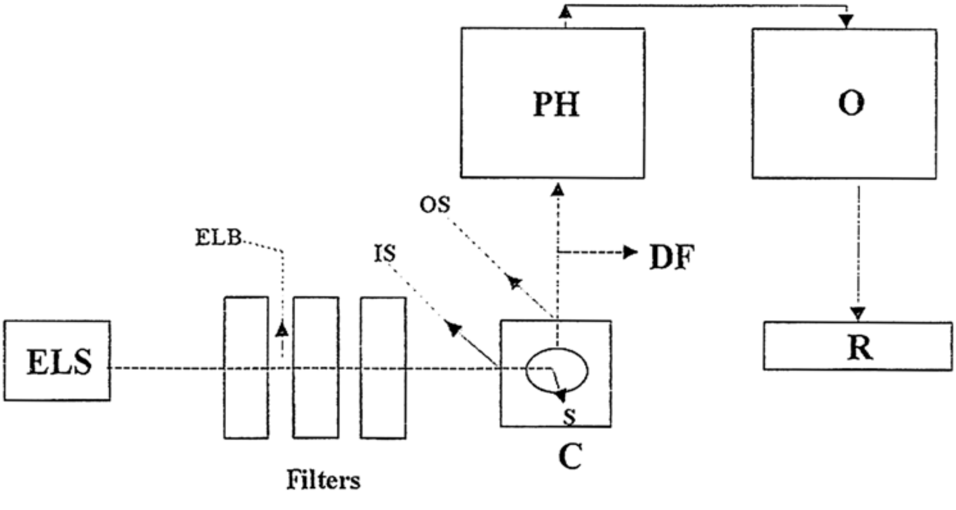

Fig. 1 — Potential scheme of the method and measuring equipment for chlorophyll delayed fluorescence: C — dark chamber with a sample stand; s — sample (leaf segment); Filters; ELS — excitation light source; PH — photo-multiplier; O — oscilloscope; R — printer; ELB — excitation light beam; DF — luminescent light; IS — input chamber slot; OS — output chamber slot.

RESULTS

The obtained experimental results on chlorophyll DF intensity changes in the function of initiation time served for further processing and as such are presented in this paper for each inbred separately.

The inbred ZPPL 14

Figure 2 shows the temperature dependence of this inbred within the temperature range of 23—58°C. The intensity of chlorophyll DF commences to increase at 27°C. The intensity increases at the temperature of approximately 30°C, and then at 34 and 47°C. The rounded maximum occurs at 48—49°C. Further temperature increase resulted in a monotonous, steep and linear descend of chlorophyll DF intensity (Figure 2).

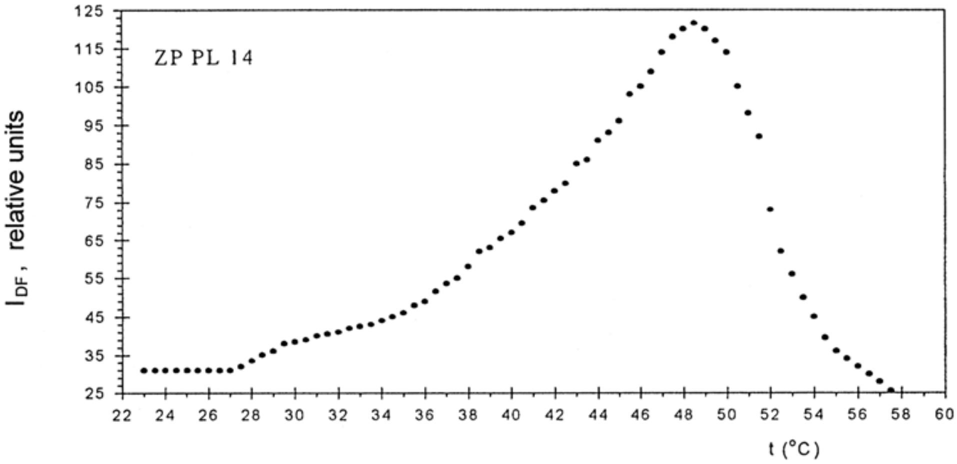

Fig. 2 — Changes of chlorophyll-delayed fluorescence intensities (I_{DF}) of thermal processes in dependence on temperature impacts in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 14

Critical temperatures at which conformational changes occurred in thylakoid membranes of inbred line ZPPL 14 were determined by the Arrhenius plot of linearization of the chlorophyll DF temperature dependence (Figure 3, Table 1). The values of critical temperatures in °C, their number and mutual distances characterize the maize inbred line in relation to its resistance to increased and high temperatures. This inbred was characterized by four temperatures (27.0, 29.5, 35.0 and 47.5°C) in the ascending part of the chlorophyll DF intensity, and two temperatures (49.5 and 53.0°C) in the descending part of the temperature dependence (Figure 3, Table 1).

Activation energies (E_a) pertaining to the ascending temperature dependence had a negative sign (—62.9, —26.1 and —61.5 kJ mol^{-1}). E_a pertaining to the descending temperature dependence showed positive values (24.2, 227.2 and 124.2 kJ mol^{-1}) (Table 1).

Tab. 1 — Changes of activation energy (Ea) and critical temperatures during thermal processes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 14

Ea, kJ/mol	t°C
—	27.0
–62.9	29.5
–26.1	35.0
–61.5	47.5
24.2	49.5
227.2	53.0
124.2	—

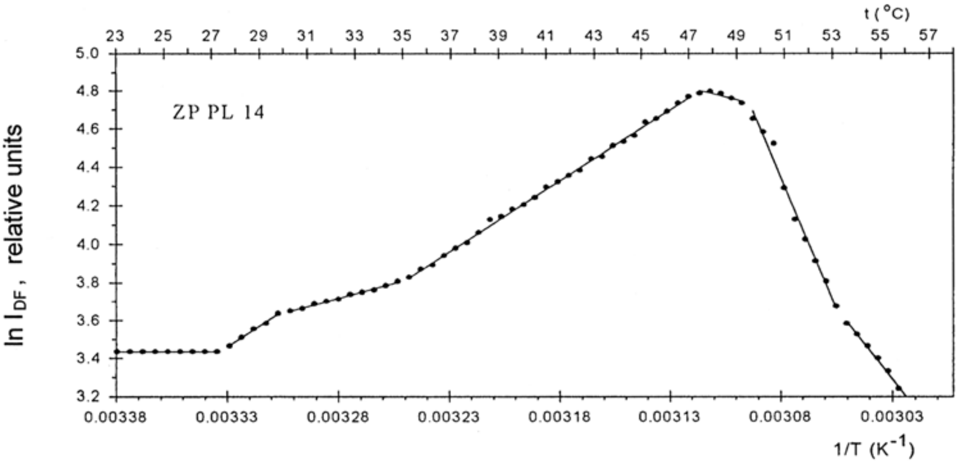

Fig. 3 — Arrhenius plot for evaluation of critical temperatures that cause conformational changes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 14

The inbred H 108

The temperature dependence of this inbred occurred initially at 32.5°C, and then a gradual increase of the chlorophyll DF intensity followed to the temperature of 49.5°C (Figure 4). The maximum intensity, not in the form of a sharp peak, occurred at approximately 50—51°C. Further temperature increase led to a steep ascent of chlorophyll DF intensity (Figure 4).

Four critical temperatures (33.0, 39.5, 44.0 and 49.5°C) were obtained by the Arrhenius plot of linearization in the ascending part (Figure 5) and only one value (51.0°C) in the descending part of the temperature dependence. Activation energies pertaining to the ascending trend had negative values (—46.3, —84.3 and —98.9 kJ mol^{-1}) (Table 2), while E_a accompanying the descending temperature dependence had positive values (7.0 and 425.8 kJ mol^{-1}) (Table 2).

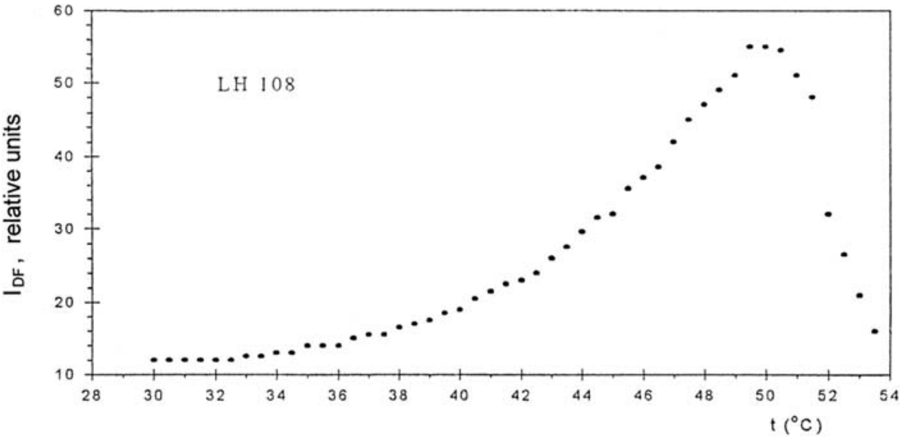

Fig. 4 — Changes of chlorophyll delayed fluorescence intensities (I_{DF}) of thermal processes in dependence on temperature impacts in thylakoid membranes of the intact leaf of the maize inbred line H-108

Tab. 2 — Changes of activation energy (Ea) and critical temperatures during thermal processes in thylakoid membranes of the intact leaf of the maize inbred line H 108

Ea, kJ/mol	t°C
—	33.0
–46.3	39.5
–84.3	44.0
–98.9	49.5
7.9	51.0
425.8	—

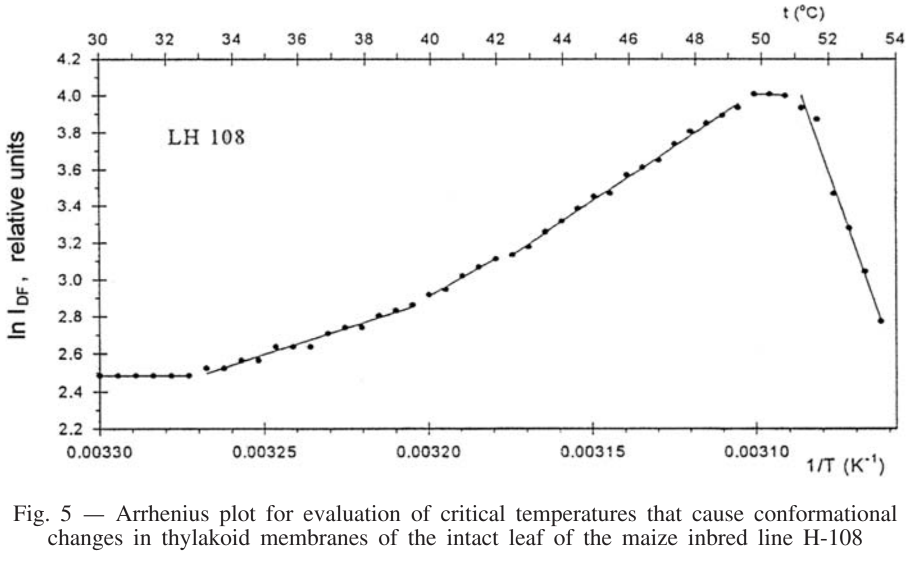

Fig. 5 — Arrhenius plot for evaluation of critical temperatures that cause conformational changes in thylakoid membranes of the intact leaf of the maize inbred line H-108

The inbred ZPPL 52

This maize inbred line underwent the same order of study — temperature dependence (Figure 6), Arrhenius plot (Figure 7), and the same processing of results on activation energies and critical temperatures (Table 3).

Tab. 3 — Changes of activation energy (Ea) and critical temperatures during thermal processes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 52

Ea kJ/mol	t°C
—	34.0
–74.41	53.0
–41.05	56.5
104.58	59.9
241.44	—

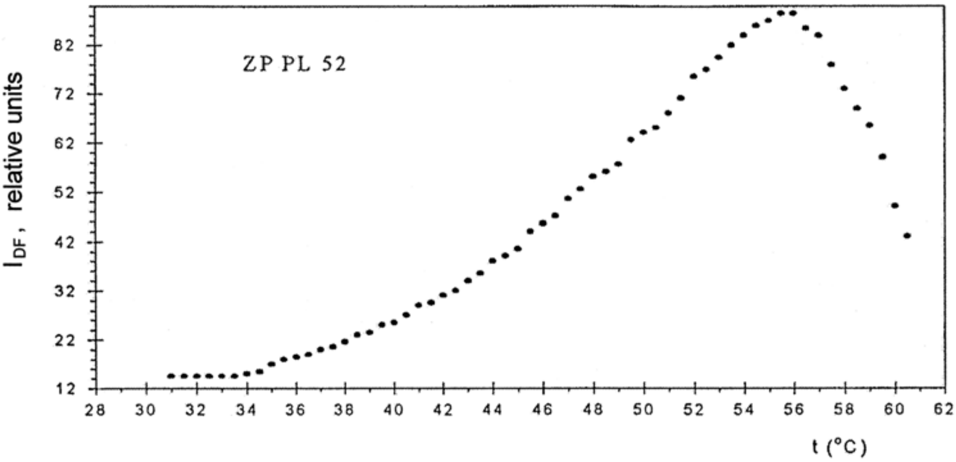

Fig. 6 — Chanres of chlorophyll delayed fluorescence intensities (I_{DF}) of thermal processes in dependence on temperature impacts in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 52

DISCUSSION

The temperature dependence of the chlorophyll DF was only partly characterized by four typical points in the investigated maize inbred lines (Figures 2, 4 and 5). The first point pertains to the lowest temperature at which the first change of the chlorophyll DF intensity was observed. The second point was related to the linear monotony and dynamics of the ascending part of the chlorophyll DF intensity. The third point expressed smaller or greater roundness of the chlorophyll DF peak intensity. Finally, the fourth point pertains to the linear monotony and dynamics of the descending part of the chlorophyll DF intensity. The stated points, although characteristic, only hinted at possible confor-

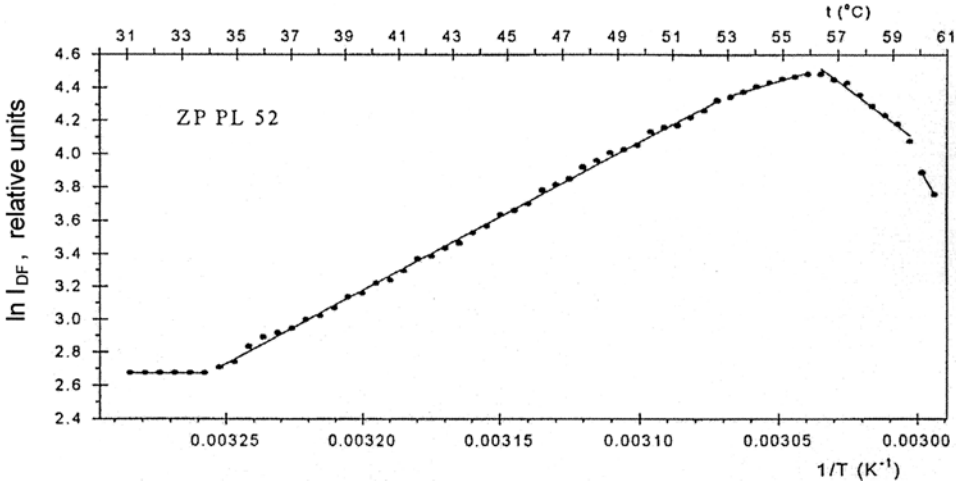

Fig. 7 — Arrhenius plot for evaluation of critical temperatures that cause conformational changes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 52

mational changes in the thylakoid membrane of the observed maize inbred lines and there are scarce literature data on the subject (Vučinić et al., 1982, Radenović, 1994, Marković et al., 1987).

All critical temperatures at which even slightest conformational changes occurred in thylakoid membranes of the studied maize inbred lines were determined by the Arrhenius plot of linearization for chlorophyll DF temperature dependence. The value of critical temperatures (expressed in °C), their number and distances characterized the maize inbred lines in relation to their resistance and adaptation to increased and high temperatures. The Arrhenius plot is based on the existence of straight lines. Each of Arrhenius straight lines represents activation energy (E_a). The intersecting points of the straight lines are determined by critical temperature. There is one value of activation energy that precedes each critical temperature, and it is followed by another value of activation energy (Radenović, 1985, 1997, Marković et al., 1993, 1996). Chlorophyll DF thermal processes in the observed maize inbred lines had activation energies with a negative sign in the ascending part of the chlorophyll DF intensity, while their values were positive in the descending part of the chlorophyll DF intensity. Such negative activation energies in thermal processes of thylakoid membranes in maize inbred lines were observed for the first time in the present study. This phenomenon is explained by the fact that the temperature increase results in smaller or greater molecular conformational changes in the thylakoid membrane, due to which molecules become more reactive and by means of that they acquire a new energy that is used in the recombination process of the chlorophyll DF occurrence.

E_a was positive in the descending part of the thermal curve, as it commonly is the case in all chemical reactions. Namely, thylakoid membranes underwent significant conformational changes, especially in the part of the thermal curve following the maximum intensity and its sharp linear decline. Such a state fits more a non-living than a living organism in relation to its structure and functions. The presence of positive E_a in the photosynthetic process originates from solar energy.

With much regret we have to state that there are no references regarding chlorophyll DF in intact leaves of higher plants that would encompass the results with parameters necessary to characterize maize inbred lines in relation to their resistance and adaptation to increased and high temperatures. The existing literature refers more to the mechanisms and processes of occurrence and development of chlorophyll DF that are of interests for biophysical, biochemical and physiological studies of the photosynthetic process (Jursinic 1986; Kerečki et al., 1986; Marković et al., 1987, 1993, 1999; Radenović 1992, 1994, 1997).

An effort was made in this study to apply the knowledge gained and the long experience for determining parameters that would make the processes of maize breeding more cost efficient. We have completed the bioluminescence method of chlorophyll DF which, being non-invasive, is not only an original but also a prospective method within the field of plant breeding.

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ЕФЕКТИ ПРОМЕНА У ТИЛАКОИДНИМ МЕМБРАНАМА — МЕРА ЗА ОЦЕНУ ОТПОРНОСТИ И АДАПТИВНОСТИ ИНБРЕД ЛИНИЈА КУКУРУЗА ПРЕМА ВИСОКИМ ТЕМПЕРАТУРАМА

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Резиме

Метод о закаснелој флуоресценцији хлорофила, као неинвазивни биолуминисцентни метод, кандидује се за примену у оплемењивању и семенарству ради утврђивања оцене инбред линија кукуруза на отпорност и адаптивност према вишим и високим температурама.

Утврђене су термалне карактеристике фотосинтетичног апарата проучаваних самооплодних линија кукуруза и то:

— температурна зависност у опсегу 23°C—60°C;

— вредности за критичне температуре на којима долази до мањих и већих структурних промена у тилакоидним мембранама,

— вредности за енергије активације (Ea, кJ/mol) дуж правих линија пре и после појаве критичне температуре у термалном процесу.

Утврђена је различита монотоност у растућем и опадајућем делу интензитета термалне криве, што указује на неједнаку отпорност и адаптираност испитиваних самооплодних линија кукуруза према вишим и високим температурама.

Проучаване самооплодне линије кукуруза у односу на њихову отпорност према вишим и високим температурама рангирају се на следећи начин:

— показано је да је самооплодна линија кукуруза ЗППЛ 52 врло отпорна према високим температурама,

— самооплодне линије кукуруза ZZPL 14 и H 108 имају задовољавајућу отпорност према високим температурама.

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EFFECT OF ECOLOGICAL FACTORS ON GENETIC SPECIFICITY FOR THE CONTENTS OF MACRO- AND MICROELEMENTS IN WHEAT GRAIN

ABSTRACT: Seven winter wheat varieties have been studied for the effect of conditions of growing on the contents of macro- and microelements in grain. Identical sets of varieties have been grown in two locations, on the chernozem soil in the vicinity of Novi Sad and on the vertisol in the vicinity of Kragujevac. The obtained results have shown that the varieties were specific with regard to the dependence of their contents of macro- and microelements in grain on the heritage, i.e., variety, ecological conditions and interactions among them. In general, the content of mineral elements in grain was specific for certain agroecological conditions, and it tended to change considerably on exposure to other agroecological conditions. Significant interactions were found between variety and location with regard to the contents of some elements.

KEY WORDS: wheat variety, grain, contents of macro- and microelements, location, interaction

INTRODUCTION

First studies on genetic specificity of plant mineral nutrition were conducted some eighty years ago (Moors, 1921). Numerous papers were published since then, dealing with this topic from various aspects and involving many crops (Vose, 1963; Klimoshevski and Chernysheva, 1980; Sarić and Loughman, 1983; Kraljević-Balalić et al., 2001). Crops and their genotypes differ significantly in the intensity of uptake, accumulation and translocation of mineral elements as well as in the tolerance to the excess or deficiency of certain elements (Kastori, 1983; Arsenijević-Maksimović et al., 2001). Genetic specificity of mineral nutrition has been studied from various aspects: interactions between variety and ecological factors and their effect on the accumulation and utilization of mineral elements (Bálint et al., 1983; Zebarth et al., 1992; Feil and Bänziger, 1993), breeding and mode of inheritance of mineral elements' content (Rasmusson et al., 1971; Mihaljev and Kastori, 1982; Kraljević-Balalić, 1996), relationships between yield on one side and nitrogen and protein contents on the other (Feil, 1998), and decontamination of soils polluted with mineral substances, primarily with heavy metals and radioactive waste (Petrović et al., 2002). Various aspects of genetic specificity of wheat mineral nutrition have been extensively studied (Petrović and Sarić, 1973; Sarić and Kovačević, 1981).

The objective of this study was to assess the effect of ecological factors on genetic specificity of mineral nutrition, i.e., the contents of macro- and microelements in wheat grain. In other words, we wanted to establish how much this characteristic depends on variety and how much on ecological factors. Taking in consideration the importance of wheat-based products in human diet and animal feeds, we wanted to improve the knowledge of the contents of biogenous elements as well as of some heavy metals, those recognized for harmful effects, in the leading Yugoslav winter wheat varieties.

MATERIAL AND METHODS

Two identical sets, each consisting of seven winter wheat varieties, were grown the same year at the experimental fields of Institute of Field and Vegetable Crops in Novi Sad and Center for Small Grains in Kragujevac.

The Kragujevac experimental field is located on the vertisol soil in the process of degradation (loessivization). The soil has a heavy mechanical composition, unstable structure and it is poor in base cations. The degree of saturation with bases is below 60%, the pH value in KCl is < 4.5. The humus content in the topsoil is medium high, with the total nitrogen content of 0.14%, the available phosphorus content of 8 mg/100 g of soil and the available potassium content of 25 mg/100 g of soil.

The Novi Sad experimental field at Rimski Šančevi is located on the chernozem soil on loess terrace. According to its mechanical composition, this soil is a loam with good, stable structure. The degree of saturation with bases is about 95%, the pH value in KCl is 7.2. The humus content is 3.5%, the total nitrogen content 0.15%, the available phosphorus content 27 mg/100 g of soil and the available potassium content of 25 mg/100 g of soil.

The climate is moderately continental in both locations. The total precipitation sum during wheat growing season is 808 m in Kragujevac and 677 mm at Rimski Šančevi.

Chemical composition of grain was analyzed after harvest. Nitrogen content was determined by the method of Kjeldahl, phosphorus by vanadate--molybdate method, potassium by flame photometry. Contents of calcium, magnesium and heavy metals were determined by AAS using a Varian AA-10.

Statistical processing was done with programs Excel 97, Statistica 5.0 and MSTAT-C.

RESULTS AND DISCUSSION

The analyzed winter wheat varieties differed considerably in their contents of biogenous macroelements (Tab. 1). Influence of location on the content of biogenous macroelements was evident. Nitrogen content was much lower in the grain produced in Novi Sad on chernozem than in the grain produced in Kragujevac on vertisol. It is difficult to explain in the light of the available data, especially when taking in consideration the nitrogen levels found in the two soil types and the fact that the agrotechnical measures applied in both locations were practically identical. Phosphorus content was higher in the grain produced on chernozem than in the grain produced on vertisol, in consequence to a lower content of available phosphorus in the latter soil type. Potassium content was slightly higher in the grain produced on chernozem. The contents of available potassium were high and identical in the two locations and this may explain why no large difference occurred between the two locations. Location appeared to have negligible effect on the contents of magnesium and calcium. In view of the low pH value and the low saturation of the adsorptive complex with bases in the case of vertisol, the contents of calcium and magnesium in the grain produced on vertisol had been expected to be lower than their contents in the grain produced on chernozem.

Tab. 1 — Effects of variety and location on the content of biogenous macroelements in wheat grain

Variety	mg % in dry matter				
	N	P	K	Mg	Ca
	Novi Sad				
Evropa 9	1,507	289.67	330.67	99.67	6.80
Milica	1,767	295.00	304.67	89.67	9.70
Renesansa	1,627	280.33	311.00	96.67	11.47
Tera	1,710	260.00	295.33	94.67	9.93
N. rana	1,713	329.33	344.00	95.67	8.07
Pobeda	1,870	360.00	322.00	100.00	8.87
Jarebica	1,610	389.33	318.00	97.00	11.20
	Kragujevac				
Evropa 9	2,040	249.33	254.67	94.00	8.27
Milica	1,900	285.33	295.33	90.67	8.80
Renesansa	2,170	324.67	357.67	105.00	10.77
Tera	2,370	299.33	326.67	104.33	10.23
N. rana	1,983	290.00	319.33	99.00	9.10
Pobeda	2,397	234.67	242.00	95.00	9.77
Jarebica	2,073	285.00	220.67	86.00	10.33
LSD 5%	0.07	11.4	31.01	3.69	0.50
LSD 1%	0.10	15.37	41.83	4.98	0.68
Location (%)	68.93	17.26	13.56	0.01	0.48
Variety (%)	17.72	25.31	30.83	45.90	82.64
Interaction (%)	9.81	55.54	41.70	43.00	13.14
Error (%)	1.75	1.89	13.90	11.10	3.74

In general, the contents of biogenous macroelements in the grain of the leading Yugoslavian winter wheat varieties were satisfactory and within the expected limits (Feil and Bänziger, 1993).

Differences were observed in the effects of variety, location and their interaction on the contents of the studied macroelements. The effect of location was most pronounced in the case of nitrogen content, the effect of variety in the case of calcium and magnesium contents. The interaction between the two factors exhibited the highest effect on the contents of potassium, magnesium and phosphorus.

The tested varieties exhibited significant differences in the contents of microelements (Tab. 2). The effect of location was pronounced. The contents of iron, zinc, nickel and cadmium were much higher in the grain produced on vertisol than in the grain produced on chernozem. The availability of these microelements is known to be higher in acid soils than in neutral or alkaline soils, which explains the results obtained. Džamić et al. (1983) found that the content of microelements in wheat grain depended to a large measure on soil pH. Similar values in the contents of manganese and lead obtained in the two locations examined in our study are difficult to explain.

Tab. 2 — Effects of variety and location on the content of microelements in wheat grain

Variety	mg/kg in dry matter						
	Fe	Cu	Zn	Mn	Ni	Cd	Pb
	Novi Sad						
Evropa 9	22.77	2.30	17.03	30.93	0.490	0.017	1.210
Milica	35.83	3.30	17.70	28.93	0.640	0.037	2.023
Renesansa	36.70	3.60	14.63	38.33	0.303	0.013	1.550
Tera	31.00	2.46	13.23	41.43	0.470	0.010	1.507
N. rana	34.43	2.63	14.76	35.20	0.633	0.057	2.247
Pobeda	31.37	2.26	15.00	36.33	0.530	0.060	2.117
Jarebica	34.93	3.30	19.20	30.70	0.500	0.050	1.930
	Kragujevac						
Evropa 9	40.30	3.80	32.26	32.03	1.000	0.123	1.353
Milica	48.67	4.26	49.80	31.03	1.240	0.107	1.373
Renesansa	43.70	6.06	54.76	30.87	0.740	0.113	1.923
Tera	46.13	5.06	52.93	30.43	0.920	0.113	1.823
N. rana	44.43	4.76	44.63	32.23	1.023	0.057	1.940
Pobeda	41.10	3.96	32.03	34.97	1.313	0.120	1.710
Jarebica	35.63	4.10	28.86	34.40	1.030	0.093	1.643
LSD 5%	3.04	0.25	1.55	8.14	0.135	0.018	0.191
LSD 1%	4.11	0.34	2.10	10.98	0.182	0.025	0.258
Location (%)	57.83	64.54	76.25	10.66	73.12	70.23	3.44
Variety (%)	23.35	25.13	9.22	34.34	18.42	5.84	54.14
Interaction (%)	14.11	9.04	14.27	50.35	3.89	19.07	33.58
Error (%)	4.71	1.29	0.26	4.66	4.57	4.87	8.84

The contents of most microelements, i.e., iron, copper, zinc, nickel and cadmium, were found to be affected by location. Exceptions were the contents of lead and manganese which were predominantly affected by variety and the interaction between variety and location.

The contents of toxic heavy metals were below the values allowed for flour in the Official Gazette (1992) — 0.1 mg/kg for cadmium and 0.4 mg/kg for lead. In the case of wheat grain, it should be borne in mind that mineral substances are predominantly accumulated in the grain coat which, depending on flour type, is either partially or completely eliminated from it.

The results of this study show that the contents of macro- and microelements in wheat grain vary in dependence of the heritage, i.e., variety, ecological conditions and interactions among them. A general statement can be made that a given genetic specificity for mineral composition in grain of wheat varieties applies only for certain ecological conditions. Changes in ecological conditions and agrotechnical practices may bring considerable changes in the contents of mineral substances in wheat grain.

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УТИЦАЈ ЕКОЛОШКИХ ЧИНИЛАЦА НА ГЕНЕТСКУ СПЕЦИФИЧНОСТ САДРЖАЈА МАКРО- И МИКРО-ЕЛЕМЕНАТА У ЗРНУ ПШЕНИЦЕ

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Резиме

Код седам сората озиме пшенице испитан је утицај услова гајења на садржај макро- и микро-елемената у зрну. Исте сорте су гајене на два локалитета, на чернозему у околини Новог Сада и на вертисолу у околини Крагујевца. Добијени резултати указују да садржај појединих макро- и микро-елемената у зрну пшенице у различитој мери зависи од наследне основе, т. ј. сорте и еколошких услова и њихове интеракције. Уопштено се може рећи да генетска специфичност сората пшенице у погледу садржаја минералних материја у зрну важи само за одређене еколошке услове. Промена еколошких услова у значајној мери мења садржај минералних материја у зрну пшенице.

Утврђене су значајне интеракције између сорте и локалитета у погледу садржаја неких елемената.

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CONCENTRATIONS OF MACRONUTRIENTS IN DOMINANT AQUATICS OF THE LAKE PROVALA (VOJVODINA, YUGOSLAVIA)

ABSTRACT: Nutrients and trace metals, accumulated in macrophyte tissues are an important indicator of water quality. In this study the concentrations of macronutrients (N, P, K, Ca) and Na in the most common submerged and floating aquatic plants, *Ceratophyllum demersum* L., *Myriophyllum spicatum* L. and *Nymphoides flava* Hill. of Lake Provala were determined. Analyses were done during the seasonal biomass peak (June—August) in two years 1996 and 1998. Chemical analyses showed significant differences in element content between the analyzed aquatics. Significantly higher ash and Ca content in submerged when compared to floating aquatic plants was found. Only small variations of investigated macroelements as related to the year of investigation, except for calcium showing significantly higher 1998 values, were recorded. The results suggest that Lake Provala belongs to β — mesosaprobic waters with moderate pollution and standard odor.

KEY WORDS: Macrophytes, *Ceratophyllum demersum* L., *Myriophyllum spicatum* L., *Nymphoides flava* Hill., elemental composition, eutrophycation, pollution

INTRODUCTION

Concentration of chemical elements in an aquatic environment is related primarily to dissolution from the sediment and also to the nature and amount of element input. Aquatic macrophytes may accumulate considerable amounts of chemical elements, nitrogen and phosphorus in particular, thus contributing to lower eutrophication, nutrient cycling, water quality control, and sediment stability (Chambers and Prepas, 1994). In addition to their role as nutrients, metals in higher concentrations and their free ions may have a toxic effect upon plant tissue. Therefore, macrophytes play an important role in nutrients cycling in an aquatic ecosystem: direct — bioconcentration and indirect — slow down of water stream flow accelerating sedimentation of dispersed particles and ions (St-Cyr et al., 1994). Element bioaccumulation depends upon plant species, its organs, and numerous abiotic factors, temperature, pH, elemental concentration in water, etc. (Lewis, 1995; Lewander et al., 1996). Their ability to accumulate chemical elements, as well as their remarkable production of organic matter in aquatic ecosystems make macrophytes „*in situ*" indicator species of water quality (Abo-Rady, 1980; Manny et al., 1991). Such a role of macrophytes is strengthen by significant interspecific variability of nutrient concentrations in plant tissues (Boyd, 1970; Gupta, 1998). Genotypic specificity, i.e. various susceptibility of plant species to the presence of chemical elements in water and sediments, also plays an important role in choosing test — plants (Dinka, 1986). Besides their ability to bioaccumulate higher concentrations of chemical elements, plants should be typical and widespread in an ecosystem under investigation.

Pollution of natural streams and lakes has a profound impact upon a need for defining plant species which might be used as indicator species of eutrophycation and pollution.

The purpose of this investigation was to determine the concentration of makronutrients (N, P, K, Ca) and Na in the most common submerged and floating aquatic plants in the Lake Provala. The survey undertaken could confirm the accuracy of the applied method for the determination of chemical composition of the aquatic macrophytes in order to evaluate the water quality.

Study area

The Lake Provala is situated in southwestern Vojvodina, (45°29' latitude and 18°86' longitude). The lake was formed during great floods in 1924. It extends from northeast to southwest, pear-like in shape with wider northern part. The lake is 390 m long, while average width of its northern rounded part exceeds 140 m. The lake receives water from subterranean streams, precipitation and floods of the Danube (Bogdanović, 1985). Its, in most part, natural development has been disturbed by human activities altering this aquatic ecosystem. By providing exceptional conditions for recreation and rest, this lake which has been stocked with fish recently deserves our concern about the impact of these cultural disturbances. The most effective way to reduce damages is to find appropriate measures for conserving natural balance of this ecosystem.

SAMPLING AND ANALYTICAL METHODS

Samples of aquatic plants: *Ceratophyllum demersum* L., *Myriophyllum spicatum* L. and *Nymphoides flava* Hill. were collected from randomly selected sites at maximum development stage and the peak seasonal biomass (June—August) during two years 1996 and 1998. Sampling sites were chosen according to their abundance from the boat. The plants were rinsed several times in tap water to remove the adherent periphyton and detritus. After final rinsing in distilled water, material was dried and prepared for analyses following Standard methods for the examination of water and wastewater (Franson, 1995).

Before chemical analysis plant material was dried at 100°C and milled. Total N concentration in the dry matter was determined by standard microkjeldahl method (Nelson and Sommers, 1973). The concentrations of P, K, Ca and Na were determined after dry ashing at 500°C and treatment with 25% HCl. Then phosphorus was assayed spectrophotometrically by ammonium-vanadate-molybdate method (Gericke and Kurmies, 1952), potassium and sodium by using a flame photometer, and calcium by atomic absorption spectrophotometer.

All analyses were carried out in three independent replications and results are given as mean values for each locality. Data were analyzed by Duncan's multiple range test, and testing was done for the level of significance p = 0.05.

RESULTS

The obtained results point to a specific ash content and also specific content of certain investigated elements in plant tissues (Tab. 1). Despite the recorded differences in absolute ash content in 1996 and 1998, similar trends may be quoted. The highest average (two years) content of total ash was found in *M. spicatum* (31.80%), then in *C. demersum* (27.37%) whereas the lowest in *N. flava* (11.45%). Most of total ash was dissolved while the remaining undissolved ash — sedimentary matter like mud and sand, although less abundant (6.64—1.03%) also had a significant share in total ash content.

Tab. 1 — Ash content in aquatic macrophytes of Lake Provala during two years (in %)

	1996			1998		
	total ash	dissolved ash	undissolved ash	total ash	dissolved ash	undissolved ash
Ceratophyllum demersum	23.58^{b}	17.07^{b}	6.54^{a}	31.17^{a}	27.30^{a}	3.87^{a}
Myriophyllum spicatum	32.62^{a}	25.97^{a}	6.64^{a}	30.98^{a}	26.60^{a}	4.38^{a}
Nymphoides flava	8.46^{c}	7.43^{c}	1.03^{b}	14.45^{b}	12.34^{b}	2.11^{b}
LSD 5%	4.41	3.23	1.45	3.21	1.69	1.68

Values with the same letter superscripts do not differ significantly at p = 0.05 level

Only small variations of investigated macroelements as related to the year of investigation, except for calcium showing significantly higher 1998 values, were recorded (Tabs 2a, 2b).

Tab. 2 — Macronutrients and Na content in aquatic macrophytes of Lake Provala during two years (in %)

a

1996	N	P	K	Ca	Na
Ceratophyllum demersum	1.79^{a}	0.17^{c}	1.71^{a}	3.08^{b}	0.20^{a}
Myriophyllum spicatum	1.51^{b}	0.20^{b}	0.55^{c}	9.18^{a}	0.17^{b}
Nymphoides flava	1.77^{a}	0.25^{a}	1.47^{b}	0.70^{c}	0.15^{c}
LSD 5%	0.06	0.02	0.19	0.60	0.02

b

1998.	N	P	K	Ca	Na
Ceratophyllum demersum	2.02^{a}	0.21^{b}	1.84^{a}	9.58^{a}	0.23^{a}
Myriophyllum spicatum	1.42^{c}	0.11^{c}	0.53^{b}	10.00^{a}	0.19^{c}
Nymphoides flava	1.89^{b}	0.23^{a}	1.88^{a}	2.91^{b}	0.20^{b}
LSD 5%	0.07	0.02	0.11	0.57	0.01

Values with the same letter superscripts do not differ significantly at p = 0.05 level

Intergenus specificity in **nitrogen** accumulation was evident. Significantly the highest nitrogen content was found in *C. demersum*, whereas the lowest in M. spicatum. In 1996 smaller variations of **phosphorus** content among the aquatics ranging from 0.17% in *C. demersum* to 0.25% in *N. flava* were obtained. In 1998, the lowest phosphorus was recorded in *M. spicatum* (0.11%), whereas the highest again in *N. flava* (0.23%). During two years of investigation and as related to plant species, the smallest **potassium** content was in *M. spicatum* (0.55 and 0.53%, respectively). In *C. demersum* inconspicuous variations of potassium content as related to year of investigation were found (1.71 in 1996 and 1.84% in 1998). The highest **calcium** was found in *M. spicatum* showing no significant variations as dependent upon year of investigation (9.18—10%). In the remaining two aquatics however, in 1998 Ca content was even 3.5 times higher. Regardless of the variations related to year of investigation, rather similar calcium distribution as related to plant species is found, i.e. two submerged plant species *C. demersum* and *M. spicatum* showed higher Ca accumulation due to photosynthesis and respiration in the aquatic environment when produced $CaCO_3$ is deposited on plants. Similar **sodium** concentrations in 1996 and 1998, amounting about 0.2% were recorded. A remarkable intergenus specificity was observed thus pointing to *C. demersum* where the highest sodium accumulation was recorded.

Higher concentrations of the investigated elements were found in mud from a deeper — northwestern part of the lake (Tab. 3). NO_3^- concentration was higher in deeper water. High calcium concentrations were found in both mud and water irrespective of water depth. Also, sodium concentrations in mud and water were not related to water depth while its mud values were 10 times those of water.

Tab. 3 — Content of macronutrients in water and mud of Provala lake

a.

water	NH_4^+	NO_3^-	PO_4^{3-}	Ca	Na
	$\mu g\ ml^{-1}$			%	
south-east	0.17	0.15	0.054	0.80	0.09
north-west	0.15	0.26	0.058	0.75	0.09

b.

mud	P	K	Ca	Na
	%			
south-east	0.05	0.05	6.75	0.10
north-west	0.11	0.10	6.50	0.11

DISCUSSION

In order to define distinct ecological conditions of a habitat, the photosynthetic vascular plants — macrophytes can be used as test-organisms. Aquatics may be used as indicator species of water quality either through their temporal and spatial distribution or by using chemical analyses to determine the content of the individual elements in their tissues (Whitton, 1979; St-Cyr and Campbell, 1994).

The Lake Provala is dominated by *C. demersum*, *M. spicatum*, and *N. flava* therefore imposing their role as bioindicators of water quality. *C. demersum* and *M. spicatum* are submerged macrophytes showing greater ability of nutrient bioaccumulation. A floating species *N. flava* is occasionally found in great populations. It is an indicator species of still low eutrophication while the presence of *Cladophora sp.* points to possible accelerated changes. The Lake Provala belongs to β — mesosaprobic waters with moderate pollution and standard odor. Such characteristics are in accordance with the chemical composition of dominant macrophytes where the highest nitrogen concentration recorded in *C. demersum* amounted 2%. Extremely high calcium concentrations in plants (up to 10%) and its significantly elevated concentrations in 1998 show its increased water concentrations concurrent with accelerated photosynthesis and respiration processes resulting in an increased biomass accumulation. Here also a significant role of the aquatic vegetation in water purification due to the absorption of great amounts of water nutrients may be emphasized.

Content of the nutrients and trace metals in plants from the littoral zone of Lakes Balaton (Hungary) and Srebarna (Bulgaria) was significantly smaller then in our investigated aquatics (Kochev and Yurukova, 1996; Yurukova and Kochev, 1996). Irrespective of the fact that the Lake Provala is a closed aquatic ecosystem, an agreement of our data with those obtained for the aquatic vegetation of DTD canal network (Stojanović et al., 1994) may be quoted.

In our opinion, above investigations offer promise as a useful framework for developing techniques for the evaluation of nutrient concentrations in natural ecosystems (Stanković et al., 2000). Further monitoring of parameters of the Lake Provala are needed for a fuller understanding of how to solve the problems of conservation of this ecosystem.

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КОНЦЕНТРАЦИЈА МАКРОЕЛЕМЕНАТА У ДОМИНАНТНИМ АКВАТИЧНИМ МАКРОФИТАМА ЈЕЗЕРА ПРОВАЛА (ВОЈВОДИНА, ЈУГОСЛАВИЈА)

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Резиме

Концентрације макроелемената и тешких метала у ткиву акватичних макрофита представљају важан индикатор хемијског квалитета воде. У овом раду испитиване су концентрације азота, фосфора, калијума и калцијума, те корисног елемента натријума у ткиву доминантних субмерзних врста *Ceratophyllum demersum* L. и *Myriophyllum spicatum* L. и флотантне врсте *Nymphoides flava* Hill. у језеру Провала у Војводини. Хемијске анализе су рађене током две године, 1996. и 1998, у летњем периоду (јун—август) када је присутна највећа органска продукција. Добијени резултати су указали на значајну разлику у концентрацији испитиваних елемената између анализираних врста. Субмерзне врсте су имале сигнификантно већу концентрацију калцијума у односу на испитивану флотантну врсту. У односу на годину истраживања констатоване су мале разлике у концентрацији елемената у ткиву биљака, изузев у односу на калцијум. Концентрације калцијума су у све три испитиване врсте биле веће у 1998. години.

На основу добијених резултата може се рећи да вода језера Провала припада β — мезосапробном типу умереног загађења и стандардног мириса.

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ENDEMIC SPECIES IN FLORA OF ST IVAN HILL IN THE VICINITY OF THE CITY OF KOTOR (MONTENEGRO, YUGOSLAVIA)

ABSTRACT: In flora of the City of Kotor twenty endemic plant species out of which even eighteen are endemics of the Balkan Peninsula (*Iris pseudopalida* Trinajstić, *Micromeria dalmatica* Bentham, *Acinos majoranifolius* Mill., *Asperula scutelaris* Vis., *Rhamnus intermedius* Steud. et Hochst, *Seseli globiferum* Vis., *Stachis menthifolia* Vis., *Fritilaria gracilis* (Ebel) Ascherson & Graebner, *Moltkia petraea* (Tratt.) Griseb., *Chaerophyllum coloratum* L., *Satureja montana* L. ssp. *illyrica* Nyman, *Cerastium grandiflorum* Waldst. & Kit, *Arum petteri* Schott, *Vincetoxicum huteri* Vis. & Ascherson, *Galium firmum* Tausch, *Euphorbia glabriflora* Vis., *Petrorhagia glumacea* (Chaub. & Bory) P. W. Ball. et Heywood), and *Dianthus ciliatus* Guss. subsp. *dalmaticus* (Celak) Hayek, one the Balkan endemic species with an exclave in southern Italy (*Portenschlagiella ramosissima* (Portenschl.) Tutin, and a Balkan-Apennine species (*Campanula ramosissima* Sibth. & Sm.) were recorded. The area surrounding the City of Kotor is locus classicus of three of the above species (*Seseli globiferum* Vis., *Stachis menthifolia* Vis., and *Micromeria dalmatica* Bentham).

All the mentioned endemic species are rare not only in the surveyed region, but, also, in flora of the Balkan Peninsula and Europe.

KEY WORDS: Flora, endems, distribution

INTRODUCTION

The City of Kotor is situated at the foot of the Lovćen Mountain spures in the southeastern-most part of the Boka Kotorska Bay. St Ivan Hill (elevation 260m) raising in the east of the City is separated from the Lovćen Mountain mass and enclosed by high cliffs and deep chasms.

Geologically, St Ivan Hill is mostly composed of Mesozoic calcareous mass coming to the surface in the form of calcareous rocks and covering most of the Hill. The remaining part is covered with a thin layer of loose humus-sandy soil in the fissures of rocks (Luković, 1953).

Climate of the City of Kotor is characterized by the Mediterranean influence with mild and rainy winters and dry summers (Lipovac, 1976). On the average, this region is 277 days without wind. The most frequent during a year is south wind (which blows toward land) while winter wind is northeast wind (which blows toward the sea). In summers, mistral which is substituted by the wind blowing towards the sea moderating summer heat blows in the morning and in the early afternoon (Žunjić, 1976/1977).

With respect to vegetation, as remnants of former evergreen Mediterranean forest, dominant are maquis and pseudomaquis (Herman, 1971).

The St Ivan Hill is the area where the remnants of old fortress and ramparts in which the City is enclosed are found (Luković, 1953).

Visiani, Baldacci, Tomassini, Pantochek, Studniczka, Horvatić, Pulević, and Šilić were among numerous botanists who surveyed the flora of this region during last and the beginning of this century.

MATERIAL AND METHODS

Plant material was collected in the period 1994—1996 and herbarized in the Herbarium of the Institute of Biology, Novi Sad (Yugoslavia) (BUNS) and Herbarium of the Institute of Botany and Botanical Garden „Jevremovac", Belgrade (BEOU). It was determined according to *Flora Europaea* (Tutin et al. eds., 1964; Tutin et al. eds. 1968—1980), *Flora SR Srbije* (Josifovic, M. ed. I—IX, 1970—1976), and *Nuova Flora Analitica d'Italia* (Fiori, 1969), *Flora d'Italia* Vol. I—III (Pignatti, 1982), whereas the species *Iris pseudopalida* after Trinajstić (1976). Plant synonimic was completely adapted to *Flora Europaea* (Tutin et al. eds., 1964; Tutin et al. eds. 1968—1980) and to the papers of Trinajstić (1976) and Šilić (1979).

REVIEW OF THE ENDEMIC SPECIES OF FLORA OF ST IVAN HILL

Iris pseudopalida Trinajstić

The City of Kotor, St Ivan Hill, May 5,1995, Budak V., Mačić V., Mačić Lj., Anačkov G. (Buns).

The species with very narrow distribution range in coastal Dalmatia, Montenegro (Trinajstić, 1976; Šilić, 1984), and Hercegovina. Occurs at dry calcareous rocky substratum and rocky pastures up to 600m elevation (Šilić, 1984). *Locus classicus* the Pelješac Peninsula (Trinajstić, 1976).

Balkan Illyrian-Adriatic endemic species (Šilić, 1984).

Micromeria dalmatica Bentham

In the vicinity of the City of Kotor, sub. *Calamintha origanifolia* Vis. (Visiani, 1847); nearby the area of the City of Kotor (Rohlena, 1942);

in the vicinity of the City of Kotor (Šilić, 1990); the City of Kotor, St Ivan Hill, August 6, 1995, Budak V., Mačić V., Mačić Lj., and Anačkov, G. (Buns).

Occurs in Bulgaria, Greece, and Yugoslavia (Chater and Guinea, 1972). Rare in central and southern Dalmatia and the Orijen—Lovćen mountain mass in Montenegro throughout (Šilić, 1984); open calcareous rocky substratum, ramparts, along roads. Locus classicus in the vicinity of the City of Kotor (Šilić, 1984).

Balkan endemic species (Chater and Guinea, 1972); western Balkan endemic (Šilić, 1984).

Acinos majoranifolius (Mill.) Šilić

The St Ivan Hill above the City of Kotor, sub. *A. adscendens* Moench M. (Visiani, 1847); the City of Kotor, St Ivan Hill, July 8,1994 and June 9,1995, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Widespread in southeastern Europe (Ball and Getliffe, 1972), in Croatia, Dalmatia, Bosnia and Hercegovina, Montenegro, Serbia, Bulgaria, Macedonia, and Albania (Hayek, 1929). Occurs at calcareous rocky substratum, edges of karst forests, along roads (Šilić, 1984).

Western Balkan and Illyrian endemic species (Šilić, 1984).

Asperula scutelaris Vis.

St Ivan Hill above the City of Kotor (Visiani, 1850; Rohlena, 1942; Baldacci In Vincek and Pulević, 1991); the City of Kotor, ramparts, August 8, 1990, Jovanović (Beou); the City of Kotor, St Ivan Hill, July 8,1994, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS); the City of Kotor, ramparts, July 13,1995, Jovanović (BEOU).

Occurs in Yugoslavia, Albania, and possibly in Rumania (Ehrendorfer and Krendl, 1976), Croatia, Dalmatia, Bosnia and Hercegovina, Montenegro, Serbia, and Albania (Hayek, 1929; Šilić, 1984) on calcareous and calcareous-dolomite rocky substrata of Mediterranean, Mediterranean-montane, and Submediterranean regions (Šilić, 1984).

Illyrian-Adriatic endemic species (Šilić, 1984).

Rhamnus intermedia Steud. et Hochst

The City of Kotor, St Ivan Hill, July 8,1994 and May 28,1995, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Croatia (Istria and Dalmatia), Bosnia and Hercegovina, Montenegro, and Albania in the holm oak (*Q. ilex*) zone at rocky terrains (Hayek, 1927; Šilić, 1984) in maquis and pseudomaquis.

An endemic species of coastal Dinaric Alps (Šilić, 1984).

Seseli globiferum Vis.

The City of Kotor (Visiani, 1850; Šilić, 1984); the City of Kotor, ramparts, July 5, 1990, Jovanović, Lakušić, and June 13, 1995, Jovanović (BEOU); the City of Kotor, St Ivan Hill, July 16, 1994, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Dalmatia, Montenegro (Hayek, 1927), and Hercegovina (Šilić, 1984), in sunny habitats, in fissures in calcareous and calcareous-dolomite rocks up to 1 000m elevation. *Locus classicus* in the vicinity of the City of Kotor (Visiani, 1850).

Endemic species of southeastern Dinaric Alps (Šilić, 1984).

Stachys menthifolia Vis.

The City of Kotor, fortress (Visiani, 1847); St Ivan Hill (Tomassini In Vincek and Pulević, 1991); the City of Kotor, ramparts, August 10,1990, Jovanović (BEOU); the City of Kotor, St Ivan Hill, July 16, 1994, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Dalmatia, Montenegro, and Albania (Hayek, 1927; Ball, 1972; Šilić, 1984), in karst forests and underbush, forest edges in Mediterranean and Mediterranean-montane belt (Šilić, 1984). *Locus classicus* in the vicinity of the City of Kotor (Visiani, 1847).

Endemic species of southeastern Dinaric Alps (Šilić, 1984).

Fritillaria gracilis (Ebel) A. & G.

The City of Kotor, St Ivan Hill, April 20,1994, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Croatia (Dalmatia), Bosnia and Hercegovina, Montenegro, and northern Albania (Hayek, 1933; Šilić, 1984), scattered or in small groups in termophilous forests and underbushes on calcareous substratum (Šilić, 1984).

Endemic species of Dinaric Apls (Šilić, 1984), rare, conserved in Montenegro (Bulić ed., 1995).

Moltkia petraea (Tratt.) Griseb

On caves in the vicinity of the City of Kotor (Studniczka In Hirc, 1906); the City of Kotor, St Ivan Hill, April 26, 1994, May 10, 1995, and August 17, 1995, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Dalmatia, Bosnia and Hercegovina, Montenegro, and Albania (Hayek, 1927; Šilić, 1984), with a disjunction in Greece; in sunny habitats, on calcareous substratum in Mediterranean, Mediterranean-montane, and Submediterranean belt (Šilić, 1984).

Endemic species of central and southeastern Dinaric Alps (Šilić, 1984).

Chaerophyllum coloratum L.

The City of Kotor, St Ivan Hill, May 1, 1994 and May 25, 1995, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Croatia, Bosnia and Hercegovina, Montenegro, Albania (Hayek, 1927; Šilić, 1984), and Serbia (Kosovo) (Nikolić, 1973; Šilić, 1984), on rocky, skeletal, and extremely degraded habitats, in barren gardens, vineyards, and olive groves (Nikolić, 1973; Šilić, 1984).

Endemic species of Dinaric Alps (Šilić, 1984).

Satureja subspicata Bartl subsp. *subspicata*

Hills surrounding the City of Kotor, sub. *S. montana* L. var. *subspicata* Bartl. (Visiani, 1847), sub. *S. pygmaea* RCHB. (Schlosser, Vukotinović, 1869).

Occurs in Croatia, Bosnia and Hercegovina, Montenegro, and northern Albania, with a disjunction in Serbia (the Tara Mountain), at windy sites on calcareous and dolomite substrata (Šilić, 1979).

Western-Balkan Illyrian endemic species (Dinaric Alps endemic species) (Šilić, 1984).

Cerastium grandiflorum W. et K.

Hills surrounding the City of Kotor (Visiani, 1850).

Occurs in Croatia, Bosnia and Hercegovina, Serbia, Montenegro, and Albania (Hayek, 1927; Gajić, 1970; Domac, 1973; Trinajstić, 1978; Šilić, 1984), in fissures in calcareous rocks in sunny habitats of montane and Subalpine belt (Šilić, 1984). Endemic species of Dinaric Alps (Šilić, 1984).

Hayek (1927) quoted this plant species from Bulgaria, whereas no record in *Flora Reipublicae Popularis Bugaricae* has been found (Вълев, 1966). Domac (1973) described it for Macedonia and Slovenia. Trinajstić (1978) also quoted this species from Slovenia, whereas no description in *Mala Flora Slovenije* was given (Martinčič, Sušnik, 1984).

Arum petteri Schott.

The City of Kotor, St Ivan Hill, May 1, 1994, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Croatia, Bosnia and Hercegovina, Montenegro, and northern Greece (Hayek, 1933; Domac, 1973; Šilić, 1984), in lowland and montane belt of the coastal karst on calcareous substratum (Šilić, 1984).

Western Balkan (Illyrian) endemic species (Šilić, 1984).

Vincetoxicum huteri Vis. et Aschers.

The City of Kotor, St Ivan Hill, July 8, 1994, August 17, 1994 and June 9, 1995, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Croatia (Hayek, 1927; Domac, 1973), Bosnia and Hercegovina, Montenegro, Albania (Hayek, 1927; Šilić, 1984), Macedonia (Šilić, 1984), and Serbia (Kosovo) (Diklić, 1977) as *Cynanchum huteri* (Vis. et Asch.) Pittoni ex K. Schum, at rocky terrains, sunny sites, moderately acid, humus soils (Šilić, 1984).

Western Balkan endemic species (Šilić, 1984).

Galium firmum Tauch.

The City of Kotor, St Ivan Hill, June 4, 1994 and June 9, 1995, Budak V., Mačić V., Mačić LJ., Anačkov G. (BUNS).

Occurs in Serbia (Hayek, 1927; Gajić, 1973; 1976; Nikolić et al., 1986), Dalmatia, Montenegro, Bulgaria, Macedonia, Albania, Tesalia, Greece, Crete Islands, Sorio, Kasos, and Karpathos (Hayek, 1927), in rocky and dry grass habitats (Gajić, 1973; Ehrendorfer, 1976).

Western Balkan endemic species (Ehrendorfer, 1976).

Euphorbia glabriflora Vis.

The City of Kotor, St Ivan Hill, May 25, 1996, Budak V., Mačić V., Mačić Lj., Anačkov, G. (Buns).

Occurs in Bosnia and Hercegovina, Montenegro, Serbia, Macedonia, Albania, and Tesalia (Hayek, 1927; Janković et Nikolić, 1972; Šilić, 1984), at sunny rocky terrains (Janković et Nikolić, 1972).

Endemic species of Balkan Peninsula (Smith et Tutin, 1968; Šilić, 1984).

Petrorhagia glumacea P.W. Ball. et Heywood

The City of Kotor, St Ivan Hill, and fortress surroundings: sub. *Dianthus obcordatus* Reut. et Marg. (Visiani, 1850).

Occurs in Croatia, Dalmatia, Bosnia and Hercegovina, Montenegro, and Macedonia: sub. *Kohlrauschia obcordata* Rchb. (Hayek, 1927; Domac, 1973; Trinajstić, 1979); Albania, Epirus, Tesalia, Ionic Islands, Greece, and Crete: sub. *Kohlrauschia obcordata* RCHB. (Hayek, 1927); Bulgaria (Раденкова, 1966) and Serbia (Gajić, 1977; Nikolić et al., 1986), at rocky terrains and crop edges (Gajić, 1977).

Balkan endemic species (Ball, 1964).

Dianthus ciliatus Guss. subsp. *dalmaticus* (Celak) Hayek

The City of Kotor: sub. *D. ciliatus* Guss. var. *cymosus* Vis. (Visiani, 1850); St Ivan Hill (Tomassini In Vincek and Pulević, 1991); the City of Kotor, ramparts: *D. ciliatus* subsp. *dalmaticus*, August 8, 1990, leg.et det.: S. Jovanović (BEOU); the City of Kotor, St Ivan Hill, June 18, 1994, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

From Dalmatia to Albania (Tutin, 1964), at sunny rocky terrains (Visiani, 1850).

Western Balkan-Adriatic endemic species.

Campanula ramosissima Sibth. et SM.

The City of Kotor, fortress surroundings — St Ivan Hill (Visiani, 1847); the City of Kotor, St Ivan Hill, May 7, 1994 and June 18, 1995, Budak V., Mačić V., Mačić Lj., Anačkov G. (BUNS).

Occurs in Dalmatia, Montenegro, and Macedonia (Hayek, 1927; Domac, 1973), Greece (Halacsy, 1902; Hayek, 1927), Epirus, Tesalia, and Ionic Islands (Hayek, 1927), Italy and Albania (Pignatti, 1982), at sunny rocky terrains and ramparts (Šilić, 1984).

Illyrian-Apennine endemic species.

Portenschlagiella ramosissima (Port.) Tutin

The City of Kotor (Visiani, 1850); the City of Kotor, St Ivan Hill (Tomassini In Vincek and Pulević, 1991).

Occurs in Dalmatia, Bosnia and Hercegovina, and Montenegro (Hayek, 1927; Tutin, 1968; Domac, 1973; Šilić, 1984), southern Italy (Tutin, 1968; Pignatti, 1982), and northwestern Albania (Hayek, 1927; Tutin, 1968; Šilić, 1984), at sanny habitats, in fissures in calcareous rocks in Mediterranean and Mediterranean-montane belt up to 1 000 m elevation (Šilić, 1984).

Endemic species of Dinaric Alps with an exclave in southern Italy (Šilić, 1984).

CONCLUSION

In flora of the City of Kotor surroundings twenty endemic species out of which two (*Euphorbia glabriflora* Vis. and *Petrorhagia glumacea* (Chaub. & Bory) P. W. Ball. et Heywood) Balkan, five (*Acinos majoranifolius* Mill., *Arum petteri* Schott., *Galium firmum* Tausch, *Micromeria dalmatica* Bentham, and *Vincetoxicum huteri* Vis. & Ascherson) western Balkan, namely Illyrian, eight (*Cerastium grandiflorum* Waldst. & Kit., *Chaerophyllum coloratum* L., *Fritilaria gracilis* (Ebel) Ascherson & Graebner, *Moltkia petraea* (Tratt.) Griseb., *Rhamnus intermedius* Steud. et Hoshst., *Satureia subspicata* Bartl. subsp. *subspicata, Seseli globiferum* Vis., and *Stachis menthifolia* Vis.) western Balkan, Dinaric Alps, three (*Asperula scutelaris* Vis., *Dianthus ciliatus* Guss. subsp. *dalmaticus* (Celak) Hayek, and *Iris pseudopalida* Trinajstić) Illyrian-Adriatic, one (*Portenschlagiella ramosissima* (Portenschl., Tutin) western Balkan, Dinaric Alps, with an exclave in southern Italy, and one (*Campanula ramosissima* Sibth. & SM.) western Balkan, namely Illyrian-Apennine endemic plant were recorded.

Our survey confirmed that sixteen of quoted endemic species still occur in the investigated area, whereas data on the four species (*Cerastium grandiflorum, Petrorhagia glumacea, Portenschlagiella ramosissima*, and *Satureia subspicata* Bartl. subsp. *subspicata*) go back to the XIX century.

The City of Kotor surroundings is locus classicus of three species (*Seseli globiferum*, *Stachis menthifolia*, and *Micromeria dalmatica*).

Despite the findings clearly showing that all the plant species cited above are rare not only in St Ivan Hill flora, but, also throughout Balkan Peninsula and Europe, only *Fritillaria gracillis* has been put among the conserved plant species in Montenegro.

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ЕНДЕМСКЕ БИЉКЕ У ФЛОРИ БРДА СВ. ИВАН У ОКОЛИНИ КОТОРА (ЦРНА ГОРА, ЈУГОСЛАВИЈА)

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Резиме

У флори околине Котора забележено је 20 ендемских биљака од којих су 18: *Iris pseudopalida* Trinajstić, *Micromeria dalmatica* Bentham, *Acinos majoranifolius* Mill., *Asperula scutelaris* Vis., *Rhamnus intermedius* Steud. et Hochst., *Seseli globiferum* Vis., *Stachis menthifolia* Vis., *Fritilaria gracilis* (Ebel) Ascherson & Graebner, *Moltkia petraea* (Tratt.) Griseb., *Chaerophyllum coloratum* L., *Satureja subspicata* Bartl. subsp. *subspicata, Cerastium grandiflorum* Waldst. & Kit., *Arum petteri* Schott.; *Vincetoxicum huteri* Vis. & Acherson, *Galium firmum* Tausch; *Euphorbia glabriflora* Vis., *Petrorhagia glumacea* (Chaub. & Bory) P. W. Ball. et Heywood) и *Dianthus cilitus* Guss. subsp. *dalmaticus* (Čelak) Hayek ендеми балканског полуострва, једна врста, *Portenschlagiella ramosissima* (Portenschl.) Тутин је балкански ендем са ексклавом у јужној Италији, а једна врста, *Campanula ramosissima* Sibth. & Sm. је балканско-апенински ендем. Међу наведеним ендемским биљкама за три врсте, *Seseli globiferum* Vis., *Stachis menthifolia* Vis. и *Micromeria dalmatica* Bentham, наводи се околина Котора као *locus classicus*.

Све забележене ендемске биљке у флори брда Св. Иван су и ретке биљке не само у флори испитиваног подручја, него и шире у флори Балканског полуострва па и у флори Европе.

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CONTRIBUTION TO THE KNOWLEDGE OF DISTRIBUTION OF *AZOLLA* SPECIES IN FLORA OF SERBIA

ABSTRACT: *Azolla* Lam. is a tender annual floating aquatic fern occurring in warm stagnant waters and slow streams. In 1962, it was for the first time described from the Koviljski Rit Marsh as *Azolla filiculoides* Lam. The same name was quoted in *Flora of Serbia* (1970). Subsequent findings presented in *Flora of Serbia* (1986;1992) mentioned only *Azolla caroliniana* Willd. Our survey shows *Azolla* expansion concurrent with its new localities of the Tisa river and Dunav—Tisa—Dunav (DTD) canal network (Bečej fishpond, Bečej—Bogojevo canal: Crna Bara, Srbobran; Banatska Palanka—Novi Bečej canal: Melenci, Jankov Most, Klek) where this adventive species is determined as *Azolla filiculoides* Lam.

KEY WORDS: *Azolla caroliniana* Willd., *A. filiculoides* Lam., *Flora of Serbia*, aquatic fern

INTRODUCTION

Azolla Lam. is a tender annual floating aquatic fern occurring in warm stagnant waters or slow streams. This subtropical American species growing in European botanical gardens is subspontaneously expanding. Babić and Parabućski (1962) were the first to describe the genus *Azolla* as *Azolla filiculoides* Lam. on the Balkan Peninsula. In *Flora of Serbia* (1970) Vukićević described *Azolla filiculoides* Lam. from warm stagnant waters of lowlands of Vojvodina. In *Flora of Serbia* (1986, *Appendix 2*; 1992) only a species *Azolla caroliniana* was cited while *A. filiculoides* was omitted. Vukićević (1992) quoted the following localities of *Azolla caroliniana* Willd. in the Bačka region: canals and its branches in the vicinity of Bačka Palanka, Čelarevo, Gložan, Bački Petrovac, Begeč, Futog, Rumenka, Stepanovićevo, Sirig, Bačko Gradište, Temerin, Novi Sad—Bački Jarak, Kać, Kovilj, Gardinovci, Lok, and Titel; in the Banat region: Perlez, Opovo, Čenta, and Pančevo; in the Srem region: Petrovaradin, Sremski Karlovci, Čortanovci, Susek, Banovci, and Bosut; in other parts of Serbia: Veliko Gradište (Jezero) and Smederevo. It should be emphasized that in Atlas of *Flora Europaea* (Jalas and Souminen, 1972) only the occurrence of *Azolla filiculoides* Lam. in the Vojvodina region (Yugoslavia) was quoted.

Also our investigations show that this species is expanding thus the populations of *Azolla filiculoides* Lam. have been recorded in the Tisa river and in the DTD canal network.

MATERIALS AND METHODS

Plant material was collected from a number of localities of Vojvodina in the period 1994—1999. Fresh plant material was used for determination after *Flora of Serbia* (1970; 1986; 1992), Felföldy (1990), and Rose (1981) by employing light and SE microscopy.

RESULTS AND DISCUSSION

Lamark 1785 was the first to describe the genus *Azolla* (Swenson, 1944). In older literature the genus *Azolla* was classified either into the family *Salviniaceae* (Black, 1948; Demalsy, 1953; Bailey, 1949; Benson, 1957; Mägdefrau and Ehrendorfer, 1978) or in the family *Lemnaceae* (Rose, 1981). The majority of authors states however, that the genus *Azolla* is the only genus of the family *Azollaceae* (Melchoir and Werdermann, 1954; Eichler, 1965; Moore, 1969; Vukičević, 1970;1992). The family *Azollaceae* belongs to the order *Salviniales*, class *Pteropsida* (*Filicineae*), and division *Pteridophyta*.

The genus *Azolla* includes annual freely floating ferns. Shoots 1—2 cm long with abundant dichotomous branching, small roots on lower side. Minute leaves smaller than 1mm, bright green to dark blue-green, reddish in autumn, arranged into two rows overlapping like tiles. Reddish colour of leaflets margin originates from blue-green alga *Anabaena azollae* occurring in cavities of upper leaf portion. *Azolla* indirectly benefits from the *Anabaena* ability to assimilate nitrogen gas representing its nitrogen nutrition source. Among vascular macrophytes, this nutrition mode is unique to *Azolla* only. Unicellular or bicellular pappilose hairs on adaxial leaf surface. Each leaf is divided into two parts: upper floating and assimilating and lower submerging and uptaking water. A great number of microsporangia aggregates into sori enclosed in indusia on the individual parts of lower leaves. Mature microspores are interconnected making 5—8 rounded floating massulae each containing septate or nonseptate glochidiae on its surface. Macrospores are floating due to a special air-containing body.

According to the key to the aquatic plants (Felföldy, 1990), the two *Azolla species*, *Azolla caroliniana* Willd. and *Azolla filiculoides* Lam. have been described.

Azolla caroliniana Willd. is bright green plant with acute upper leaf lobe, narrow membranous at margin and bicellular hairs at leaf surface.

Azolla filiculoides Lam. is blue-green in colour, reddish in autumn, somewhat larger than former. Upper leaf lobe obtuse, with wide membranous margin. Dense unicellular pappilose hairs on leaf surface (Fig. 1).

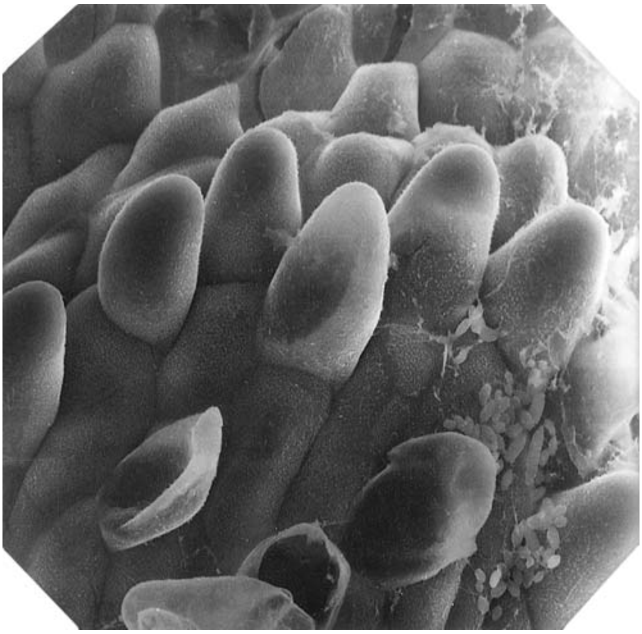

Fig. 1 — Leaf surface of *Azolla filiculoides* Lam.
with unicellular papillose hairs

In *Flora of Serbia* (ed. Josifović, 1970) the occurrence of the species *Azolla filiculoides* Lam. was quoted. Later issues of *Flora of Srbia* (ed. Sarić, 1986; 1992) however, cited only the presence of *Azolla caroliniana* Willd.

In addition to already recorded *Azolla* localities in Serbia (Vukićević, 1992), expansion of a neotropical species *Azolla filiculoides* Lam. in the Tisa, Bečej—Bogojevo canal (Crna Bara, Srbobran), Bečej fishpond, and northern Banat i.e. Banatska Palanka—Novi Bečej canal (Melenci, Jankov Most, Klek) was recorded (Fig. 3). A survey of the localities of the DTD canal network showed that *Azolla filiculoides* Lam. populations from dense green-reddish „islands" making with representatives of families *Lemnaceae* and *Salviniaceae* stands of *associations Lemno-Azolletum* Br.-Bl. 1952 and *Salvinio-Spirodeletum polyrrhizae* Slavn. 1956 of *alliance Lemnion minoris* W. Koch et Tx. 1954 ex Oberd 1957, order *Lemnetalia* W. Koch et Tx. 1954, class *Lemnetea* W. Koch et Tx. 1954 are recorded. Small dense pillow-like aggregates *of Azolla filiculoides* Lam. are found on muddy-wet sites of the Tisa.

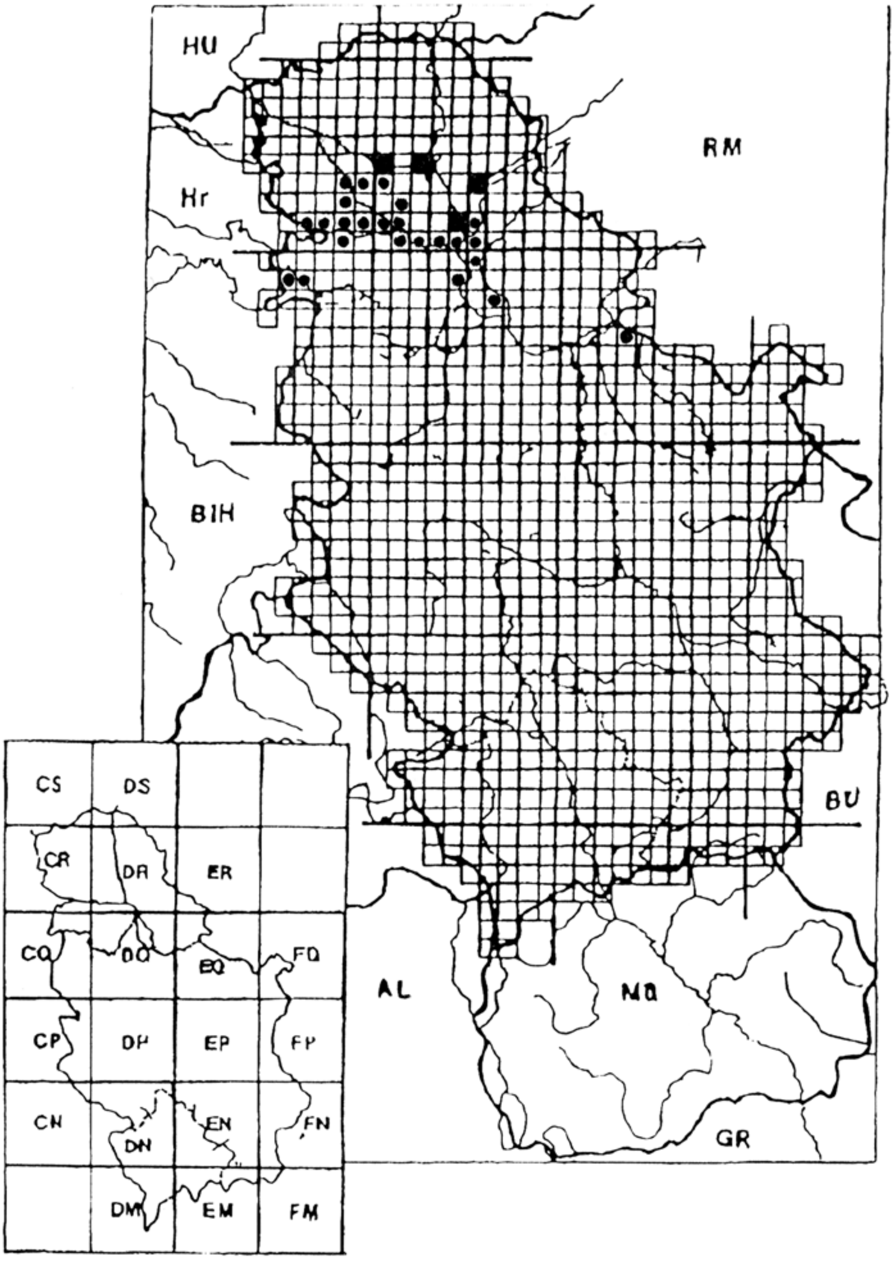

Fig. 2 — Distribution of *Azolla species* in Serbia

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ПРИЛОГ ПОЗНАВАЊУ РАСПРОСТРАЊЕЊА ВРСТА РОДА *AZOLLA* LAM. У ФЛОРИ СРБИЈЕ

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Резиме

Azolla Lam. је нежна, једногодишња, флотантна водена папрат распрострањена у топлијим стајаћим и споротекућим водама. Дихотомо је граната, с љуспастим, зеленим листовима поређаним у два реда, који се црепасто препокривају. На Балканском полуострву род *Azolla* је први пут описан 1962. године, и то као врста *Azolla filiculoides* Lam. распрострањена само у Ковиљском риту. У *Флори СР Србије* 1970. године, Вукићевић описује распрострањеност врсте

Azolla filiculoides Lam. само у топлим стајаћим водама низијског подручја Војводине. Међутим, Вукићевић, 1986. и 1992. године у *Флори Србије* описује опет само једну врсту, али сада *Azolla caroliniana*, док се присуство *A. filiculoides* не бележи. Према нашим истраживањима *Azolla filiculoides* Lam. је у експанзији, па је налазимо масовно у реци Тиси, као и у каналској мрежи ХС Дунав—Тиса—Дунав.

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INFECTIONS IN PARENTAL FLOCK AND EGGS ON *CAMPYLOBACTER* AND *SALMONELLA* KINDS

ABSTRACT: Presence of *Campylobacters* and *Salmonella* in poultry material is a matter for investigation by many authors. The results and knowledge about these two bacteria is important for poultry health protection and also for human health protection, since they are zoonosis.

The topics of our work was bacteriological investigation of digestive tract in the laying hens of parental flock and their eggs. Our aim was to isolate and identify *Campylobacter* and *Salmonella* in parental flock in order to detect findings in them and, by the ways of bacterial egg investigations, and thus contribute solving the problem concerning vertical transfer on their offspring. Also the aim of the work was to detect sensibility of the isolated microorganisms on chemioterapeutics.

For bacterial investigation we used cloak swabs from heavy lines laying hens from a house in the reproductive center where parental flocks and eggs were from the same parents. The samples were randomly taken and separated in a special cage in order to provide conditions for following special hens and their eggs. For isolating *Campylobacter* we used Columbia agar + campilosel with provided microaerophil conditions Gener box microaer, and for isolating *Salmonella* we used selenite bouillon, endo and ss (*Salmonella*-shigela) agar. Identification of both kinds was done by API strips and software reading the results, but for discovering *Salmonella* only serotipisation was used. Investigation of sensibility of the isolates on chemioterapeutics were done on the bases and in the conditions that were prescribed for isolated bacteria kinds.

A total of 50 cloak swabs and 250 eggs were examined. In laying hens *Campylobacter* kinds were isolated in 32 cases (64%), and *Salmonella* in 19 cases (38%). In the eggs there were no *Campylobacter* kinds, while out of 250 examined eggs in 33 (13.2%) *Salmonella* was discovered.

The results point on the presence of *Campylobacter* and *Salmonella* kinds in the digestive tract; in 13.2% of eggs *Salmonella* was found, but there were no bacteria from *Campylobacter* strain. Absence of *Campylobacter* kind from supports findings of those authors who consider that there is no vertical transfer of this bacteria, but findings of *Salmonella* in eggs correspond to the experience about this kind that is transported in this way. The biggest sensitivity on the isolated bacteria from *Campylobacter* kind were found towards gentamicin, oxitetracycline, ampicillin and amoxillin, and isolated on *Salmonella* stereotype towards enrofloxacine, uniquin, triquin and quinabic.

KEY WORDS: parental flock, eggs, *Campylobacter, Salmonella*

INTRODUCTION

Presence of *Campylobacter* and *Salmonella* in poultry material is a matter of investigation by many authors. The results and knowledge about these two bacteria is important for poultry health protection, but also for human health protection, since they are zoonoses. When speaking about *Campylobacter* kinds in flocks of laying hens and in eggs (Shanker, 1986), they were discovered in a flock, but not isolated in eggs. Similar findings had (Doyl, 1984) who discovered that out of 266 examined eggs, in two *Campylobacter* kind was present and it was on the surface of egg-shell, but not in its content. Presence of vibrio forms in egg follicles, oviduct and yolk in a formed egg, as well as in dead laying hens is the topics of the work (Velhner, 1980). Not enough clear fact about possible presence of *Campylobacter* kind in eggs and transfer on the offspring, as well as presence of *Salmonella*, presents a serious health and financial problem (Mrdjen, 1997) that caused this research.

The topics of our work was bacteriological investigating of digestive tract in the laying hens of the parental flock and their eggs.

Our aim was to isolate and identify *Campylobacter* and *Salmonella* in parental flock and in eggs in order to detect these kinds are present in them and to try to contribute solving the problem concerning the vertical transmission of laying hens on their offspring through eggs. In the work we are going to test microorganism sensibility on chemioterapeutics.

MATERIAL AND METHODS

For bacteriological investigation we used cloak swabs from laying hens heavy line from a building in a center with the parental flock and the eggs of same parents. The samples were randomly taken and separated in special cages in order to provide conditions for following chosen hens and their eggs. A total of 50 cloak swabs and 250 eggs were examined.

For isolation of *Campylobacter* we used Columbia agar + campilosel (antibiotic addition) and was streaked by direct technique of cloak swabs. In the case with the eggs, we streaked the egg content and parts of membrane, with provided microaerophil conditions Gener box microaer (Skirrow, M. B. and Benjamin, J., 1980, Quinn, P. J. et all., 1994). For isolating *Salmonella* we used selenit bullion with addition of the swabs and endo and ss (*Salmonella*-shigela) agar. After 48 hours of incubation at 37 C selenit bouillon was streaked again (Mrdjen, 1997). Identification of both bacteria was performed with the help of API strips and software for reading results, and in the case of *Salmonella* by the method of fast agglutination.

For investigating sensibility on hemioterapeutics for *Campylobacter* kind we used selective base nd microerophil condition as it was mentioned in isolating these bacteria, and for *Salmonella* we used Muller Hinton agar.

RESULTS AND DISCUSION

In Table 1. The results of examining 50 cloak swabs from laying hens where presence of *Campylobacter* kinds and *Salmonella* can be seen.

Tab. 1 — The results of examining 50 cloak swabs

Microbial species	Number of examined material (cloak swabs)	Number of isolates	% of positive findings
Campylobacter sp.	50	32	64,00%
Salmonela sp.	50	19	38,00%

In laying hens *Campylobacter* kinds were isolated in 32 cases which makes 64%, out of total. The results correspond to the mentioned works (Jacobs-Reitsma, 1995; Stojanov, 1999; Stojanov, 2000) where bacterial flora of digestive tract in laying hens was analyzed and the results of *Campylobacter* is within the frame of our findings. The results we got after the examination is important from proving *Campylobacter* kinds in laying hens. It may point to the possibility of vertical transmission on the offspring by eggs because this is a parental flock. This may indicate a danger for human population since this is a zoonosis.

Salmonella was isolated in 19 samples out of 50 examined which makes 38 % o positive findings. Positive findings on *Salmonella* presence is somehow higher than in the reported works (Mrdjen, 1992; Mrdjen, 1997) where poultry material was analyzed on the presence of *Salmonella*. A possible reason for this could be the origin of material, i.e. the way of raising and exploiting the animals. The findings of *Salmonella* is a danger in vertical transmission on the offspring, as well as a danger for humans in the case of consummation of the infected eggs.

When speaking about isolated bacteria, in *Campylobacter* kind we had two different species: *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. The findings of these kind in the samples correspond to the results of other authors (Doyl, 1984; Shanker, 1986; Jacobs-Reitsma, 1994a; Stojanov, 1999). This presents the most isolated strains mentioned by these authors.

From *Salmonella* kind we isolated only one serotype — *Salmonella enteritidis*. This kind of bacterial presents the main finding in the reported work (Mrdjen, 1997) where it is reported that 85.71% of positive material has its origin in poultry. This *Salmonella* serotype is the isolate most frequently found in poultry material.

Table 2 shows the results after investigating eggs on presence of *Campylobacter* kinds and *Salmonella*.

Tab. 2 — The results after investigating eggs

Microbial species	Number of examined material (cloak swabs)	Number of isolates	% of positive findings
Campylobacter sp.	250	—	—
Salmonela sp.	250	33	13,20%

In the Table 2 one can see that no *Campylobacter* kind was detected what corresponds to the results of other authors (Doyl, 1984; Shanker, 1986; Jacobs-Reitsma, 1994 a; Stojanov, 1999). Positive findings of *Campylobacter* kind in digestive tract and negative findings of these bacteria in eggs were the same as in our work and in the works of the above mentioned authors. Absence of these bacteria supports the theory that these microorganisms cannot be vertically transmitted.

Examining 250 we discovered presence of *Salmonella* in 33 samples, which is 13.2% out of total. In our investigation the finding of *Salmonella* there was only one *Salmonella* stereotype and it was *Salmonella enteridis*. This correspond to the investigation of other authors (Mrdjen, 1992; Mrdjen, 1997) when speaking about the kind of isolate and the presence of these bacteria in the material. Findings of the same *Salmonella* serotype in digestive tract and in eggs prove the possibility of vertical transmission of this bacteria and a possible danger on animals and humans.

Table 3. presents sensibility of the isolated bacteria kinds on chemioterapeutics

Tab. 3 — Sensibility of the isolated bacteria kinds on chemioterapeutics

Isolate / chemioterapeutics	Streptomicin	Gentamicin	Uniqum	Triquin	Neomicin	Amoxicill.	Linkomicin	Enrofloksac.	Flumequin	Linko.-spck.	Amoxicillin	Tribrisen	Oksitetracykl	Quinabic
Campylobacter jejuni	I	S	I	R	R	S	I	R	R	I	S	R	S	I
Campylobacter coli	I	S	S	R	R	I	I	R	I	I	S	R	S	I
Salmonella enteritidis	I	I	S	S	I	I	R	I	S	I	I	I	R	R

S — Sensitiv, I — Medium sensitive, R — Resistant

Campylobacter kinds showed sensibility towards gentamicin, amoxicilline and tetracycline, while the greatest sensibility of the isolated *Salmonella* serotype was toward uniquine, triquine, and flumequine.

Sensibility and resistance of *Campylobacter* kinds on hemitherapeutics is important from different aspects. These aspects, as it is importance of chemioterapeutics performance, should not be seen separate, but as a whole, and their division is only conditional and it is for the purpose of better understanding the problem. Knowing the drugs for treating sick animals, it presents maybe the most important reason for investigating, and our findings correspond to the investigation done by (Goldstein, 1982).

Besides the mentioned aspect, the importance of knowing isolate sensibility on chemioterapeutics, it seems that the reasons for knowing them are almost the same and possibilities are based on these characteristics, and it is isolation and diagnosis of *Campylobacter* kinds. About these facts are also the works of (Smibert, 1981; Garcia, 1983). It is well known that he isolated *Campylobacter* kind use selective bases that prevent growth of unwanted bacterial flora in the material where we wanted to prove presence of *Campylobacter*, as well as the fact that part of *Campylobacter* identification is based on already mentioned features of microorganisms.

Sensibility of *Campylobacter* and *Salmonella* towards quionolone, ampicillin, tetracycline and erythromycin is described in the work (Jacobs-Reitsma, 1994 b), but in the work of (Mrdjen, 1993) it can be seen that the greatest sensibility of *Salmonella* towards enrofloxacin, uniquine and flumequine.

CONCLUSION

1. In the work we used methodology that was used also by other investigators, so the validity of the results is obvious.
2. Findings of *Campylobacter* kind in very high percentage 64% and *Salmonella* kind up to 38% in the digestive tract indicates their presence in the samples.
3. Examining the eggs presence of *Salmonella* (up to 13.2%) was isolated, but no *Campylobacter* kinds were discovered.
4. Isolated *Campylobacter* bacteria had two strains and it were *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*, and in *Salmonella* kind *Salmonella enteritidis* was isolated.
5. Findings of *Campylobacter* kinds in the digestive tract of laying hens in the parental flock present a fact that may point to the possibility of vertical transmission of bacteria, what has consequences not only on chicken but people as well. This is true also when talking about *Salmonella* in the digestive tract in laying hens.
6. Absence of *Campylobacter* bacteria from eggs supports the opinion of the authors who think the vertical transmission is impossible. Positive finding of *Campylobacter* in the digestive tract, negative in eggs may help solving the dilemma of possible vertical transmission of these bacteria, while findings of *Salmonella* in eggs corresponds to the experience that this kind may be transmitted and thereby harm animals and people.
7. Isolate sensitivity towards hemioterapeutics is satisfactory because there is a possibility of adequate therapy in treating sick animals. The findings show that in the case of therapy, it must be done with combined medicaments, because medicaments we have used could not cover both groups of isolates.

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ИНФЕКЦИЈЕ РОДИТЕЉСКОГ ЈАТА И ЈАЈА *CAMPILOBACTER* И *SALMONELLA* ВРСТАМА

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Резиме

Налаз *Campylobacter* врста у висини од 64,00% и *Salmonella* врста са 38,00% у дигестивном тракту указује на њихово присуство у испитиваним материјалима. Прегледом јаја утврђено је присуство *Salmonella* са 13,2%, док није изолована ни једна бактерија из рода *Campylobacter*.

Изоловане бактерије из рода *Campylobacter* чиниле су две врсте и то *Campylobacter jejuni* subsp. *jejuni* и *Campylobacter coli*, а из рода *Salmonella* серотип *Salmonella enteritidis*.

Налаз кампилобактер врста у дигестивном тракту кока носиља родитељског јата представља чињеницу која може да упозори на могућност вертикалног начина преношења ове бактерије, с обзиром на производну категорију којој припадају испитиване јединке, што има своје последице како за саме пилиће тако и за људе пошто се ради о зоонози. Овакво мишљење вреди и када се ради о присуству салмонела у дигестивном тракту кока носиља.

Одсуство бактерија из рода *Campylobacter* из јаја говори у прилог мишљењу оних аутора који сматрају да није могућ вертикалан начин преношења ове бактерије.

Осетљивост изолата према хемиотерапеутицима је задовољавајућа јер постоји могућност адекватне терапије и излечења оболелих животиња. Налази су показали да се мора, када је у питању терапија, ићи на комбиновање лекова, јер лекови које смо ми користили својим дејством нису могли да покрију обе групе изолата.

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2. Preparation of manuscript

2.1. The maunscript must be typed double space throughout (including references, table, etc.), on A4 paper, and all margins should be kept wide (2.5 cm).

2.2. Manuscripts should be divided into sections, viz. Abstract, Key words, Introduction, Material and/or Methods, Results, Discussion, References, Abstract in Serbo-Croatian, Acknowledgements.

2.3. The title of the paper should contain as many relevant terms as possible, but should be limited to about 10 words.

2.4. The key words should indicate the scope of the paper. They should be given in alphabetical order and separated by commas. Key words should not exceed 100 characters.

2.5. The names, family names and middle names, of all authors with at least one first name should be spelled out for each author. The affiliation of the authors (without abbreviations) and where the contribution originated, including complete postal addresses, should be specified.

2.6. The abstract should be given in two languages, English and Serbo-Croatian. It should be as informative as possible, and it should summarize the contents of the paper. The former should not exceed 5% and the latter not 10% of the length of the entire manuscript. The abstract in Serbo-Croatian should include the title of the paper, the name(s) of the author(s), and his/their affiliation.

2.7. Acknowlegdements of financial support, advice, or other kinds of assistance should be made at the end of the paper, under the heading „Acknowledgements”.

2.8. Papers should not exceed 12 typewritten pages, including references, tables, legends, and figures.

3. References

3.1. References should be limited to those that are absolutely necessary.

3.2. References to literature should be arranged alphabetically; cite exactly as follows:

a. Journal articles

Author CD, Author DC (1990) Title of article. *Plant and Soil* 135: 102—134.

b. Book articles

Author ED, Author SI, Author BB (1991) Title of article. In: A Blom, B. Lindau, Eds., *Title of book*, Ed 3, Vol 2, Publisher, City, pp 242—255.

c. Doctoral theses, M. A. Theses and Habilitations

Author VA (1989) *Title of thesis*. PhD Thesis. University, City.

d. Books

Author AE (1987) *Title of the book*. Publishers, City, pp 237.

e. No authors or editors

Title of booklet, pamphlet, etc. (1989) Publisher of company, City.

f. Unpublished works

Cite an article as „in press” only if accepted for publication; cite the journal in which it will appear.

3.3. Journal names should be abbreviated in conformity with the Bibliographic Guide for Authors and Editors (BIOSIS, Chemical Abstracts Service and Engineering Index, Inc., 1974).

3.4. References to literature in the text should be made by mentioning the last name of the author and the year of publication. In the case of two authors, both should be mentioned, but with three or more only the name of the first author plus „et al.” should be given.

3.5. If an author is cited who published several papers in the same year, add a, b, c, etc, to the year of publication, both in the text and references.

4. Illustrations

4.1. Figures are of two kinds: black-and-white photographs and drawings. Photographs must have good contrast; line drawings must be neatly drawn, boldly in black ink on good quality white tracing paper. In addition to the usual line-drawn graphs, also treat metabolic schemes, complicated formulas, and large or complex tables as figures.

4.2. All letters, numbers, and symbols must be large enough in the original to be at least 1.5 mm high after reduction. The lettering of figures should also be drawn in black ink.

4.3. Figures should be added separately, i.e., not inserted in the typed text. Legends should, if possible, be inserted in the figures.

4.4. The position of figures should be indicated at the left margine. Figures are to be numbered with Arabic numerals.

4.5. Every figure must be accompanied by a caption. The caption must explain the contents of the figure. Captions are not to be typed under the figures, but should be compiled on a separate page.

5. Tables

5.1. Tables are to be typed on extra pages (one page per table), at the end of the manuscript.

5.2. Tables are to be numbered with Arabic numerals.

5.3. Each table must begin with a caption. The caption must explain the contents of the table.

5.4. Footnotes to a table should be typed directly under the table.

5.5. The position of tables should be indicated at the left margin.

6. Units, names, formulas, and abbreviations.

6.1. Only SI quantities and units are to be used (SI = Systeme International d'Unit's); in exceptional cases, other officially accepted units may be used.

6.2. For molar concentration, an italicized M (underlined) should be used.

6.3. Biological names in Latin should be italicized (underlined).

6.4. Chemical structural formulas and equations should be drawn (not written or typed), ready for photographic reproduction.

6.5. Only standard abbreviations should be used. Where specialized abbreviations are used, the term should be given initially in full with the abbreviation indicated in parentheses.

6.6. Mathematical expressions should be written in such a way as to use the minimal number of lines, while retaining their clarity, for example: 2/3 instead of 2:3, exp (-ab) instead of e^{-ab}, etc.

7. Short Communications

7.1. Proceedings for Natural Sciences offers an opportunity to publish short communications on all aspects that are implied by the journal's title.

7.2. Short communications are limited to 4 typewritten pages including all illustrations.

7.3. The presentation and format of the short communications are similar to those of a normal paper, except for the list of references, in which the titles of the papers should be omitted.

8. Information of authors

8.1. When the manuscript has been accepted, the author will be informed of the approximate time of publication.

8.2. Corrections of the proofs should be restricted to printer's errors only. Other than these, substantial alterations will be charged to the author. Proofs should be handled promptly and returned to the Editorial Office.

8.3. Fifty offprints are supplied free of charge. Copies in addition to these may be ordered and paid for through the Editorial Office.

9. DISKETTES: After acceptance, the final revision should be submitted on disk. Include text, tables and figures on a double-density or high-density 3.5-inch diskette. An accompanying printout is needed to facilitate the incorporation of electronic tables and figures. Word for Windows (any version) is the preferred word-processing program. When copying the paper on disk, it is important to follow this procedure: *File>Save as>Options>Embed True Type fonts>ok>Save*.

УПУТСТВО ЗА АУТОРЕ

1. Опште напомене

1.1. Зборник за природне науке прима оригиналне радове и прегледне чланке као и кратка саопштења из свих научних области које обухвата назив часописа. Прегледни радови се објављују само на позив редакције. Радови који су већ објављени или су послани за објављивање у другом часопису не могу бити прихваћени.

1.2. Прихватају се рукописи писани на енглеском језику. Језик мора бити исправан у погледу граматике и стила. Аутори треба да предају рукопис у три примерка (оригинал и две копије). Аутори чији матерњи језик није енглески такође треба да приложе и копију рада на изворном језику.

1.3. По примању рукописа, аутори ће добити редни број свога рада. Тај број треба наводити у даљој преписци. Редакција ће обавестити ауторе о приспећу рукописа и мишљењу рецензената у року од три месеца од пријема. Сваки рад рецензирају најмање два рецензента. Ако рад не буде прихваћен, рукопис се не враћа аутору.

1.4. Рукописе за објављивање треба слати на адресу редакције Зборника за природне науке, 21000 Нови Сад, Ул. Матице српске 1, Југославија.

2. Припрема рукописа

2.1. Рукописи се куцају са двоструким проредом у свим деловима текста (укључујући литературу, табеле итд.), на папиру формата А4. Све маргине треба да буду широке 2,5 сантиметра.

2.2. Рукопис треба поделити на: Сажетак, Кључне речи, Увод, Материјал и/или методе, Резултати испитивања, Расправа, Литература, Сажетак на српско-хрватском језику, Захвалност.

2.3. Назив рада треба да буде информативан, али не дужи од десет речи.

2.4. Кључне речи треба да указују на целокупну проблематику истраживања. Треба их навести абецедним редом и одвојити зарезима. Кључне речи не треба да пређу сто словних знакова.

2.5. Треба навести презимена, средње слово и имена аутора рада као и назив установе (без скраћеница) у којој је рад настао, заједно са пуном поштанском адресом.

2.6. Сажетак, на енглеском и српском, треба да буде информативан и да резимира садржај рада. Дужина енглеског сажетка треба да буде до 5%, а српског до 10% дужине укупног текста. Српски сажетак треба да садржи наслов рада, презимена и имена аутора и назив установе у којима су аутори запослени.

2.7. Податке о финансијској помоћи, саветима и другим врстама помоћи треба навести на крају рада, под насловом Захвалност.

2.8. Радови не смеју бити дужи од 12 куцаних страна, укључујући литературу, табеле, легенде и слике.

3. Литература

3.1. Литературу треба ограничити на неопходан број навода.

3.2. Литературне наводе треба сложити абецедним редом, на следећи начин:

а. Чланци из часописа Аутор CD, Аутор DC (1990) Назив рада. Име часописа 135: 102—134.

б. Чланци из књига

Аутор ED, Аутор SI, Аутор BB (1991) Назив цитираног дела књиге. У: A. Blom, B. Lindau, Eds., Назив књиге, Ed 3, Vol 2, Издавач, Град, 242—255.

в. Дисертације

Аутор VA (1989) Назив тезе. Докторска дисертација. Универзитет, Град.

г. Књиге

Аутор AE (1987) Назив књиге, Издавачи, Град, 237.

д. Публикације без аутора или уредника

Назив књиге, брошуре, итд. (1989) Издавач или установа, Град.

ђ. Необјављени радови

Навод „у штампи” треба да се односи само на прихваћене радове; навести име часописа у којем ће рад бити објављен.

3.3. Имена часописа треба скраћивати према „Bibliographic Guide for Authors and Editors” (BIOSIS, Chemical Abstracts Service and Engineerings Index, Inc., 1974).

3.4. Референце у тексту треба да укључе презиме аутора и годину издања. Ако има два аутора, треба навести обојицу, а у случају три или више аутора треба навести првог аутора и назначити „et al.”.

3.5. Ако се наводе два или више радова истог аутора, објављених у истој години, потребно је у тексту и списку литературе ставити a, b, c итд. иза године објављивања.

4. Илустрације

4.1. За илустрације могу се користити црно беле фотографије и цртежи. Фотографије треба да имају добар контраст а цртежи треба да буду цртани тушем, на папиру доброг квалитета. Осим графикона, метаболичке шеме, компликоване формуле и велике или компликоване табеле такође треба третирати као слике.

4.2. Сва слова, бројке и симболи треба да буду довољно велики у оригиналу, тако да после смањивања не буду мањи од 1,5 mm. Текст на сликама и графиконима такође треба исписати тушем.

4.3. Илустрације треба приложити уз рад а не уметнуте у текст. По могућности, легенде треба назначити на илустрацијама.

4.4. Места илустрација треба означити на левој маргини, арапским бројевима.

4.5. Свака илустрација треба да има текст који објашњава садржај прилога. Текст за илустрације треба куцати на посебној страни.

5. Табеле

5.1. Табеле треба куцати на одвојеним странама (једна табела по страни) и приложити их на крају рада.

5.2. Табеле се означавају арапским бројевима.

5.3. Свака табела треба да почне насловом који објашњава њен садржај.

5.4. Напомене треба куцати одмах испод саме табеле.

5.5. Места табела у тексту треба означити на левој маргини.

6. Јединице, имена, формуле и скраћенице

6.1. Треба користити SI ознаке количина и јединица (SI Systeme International d'Unit's), изузетно се могу користити и друге званично прихваћене јединице.

6.2. Моларну концентрацију треба означити са M и подвући.

6.3. Биолошка имена на латинском треба подвући.

6.4. Хемијске структурне формуле и једначине треба нацртати (не исписивати или куцати) и припремити за фотографску репродукцију.

6.5. Прихватају се само стандардне скраћенице. При коришћењу специјалних скраћеница, пун термин треба навести приликом првог спомињања, а скраћеницу додати под наводним знацима.

6.6. Математички изрази треба да буду написани тако да се користи најмањи број редова, али да се сачува читљивост, нпр. 2/3 уместо 2:3, exp (-ab) уместо a-ab, итд.

7. Кратка саопштења

7.1. Зборник за природне науке нуди могућност објављивања кратких саопштења о свим научним областима обухваћеним називом часописа.

7.2. Величина кратког саопштења је ограничена на 4 куцане стране, укључујући све илустрације.

7.3. Кратко саопштење се пише по упутствима за припрему рада нормалне дужине, сем што у литератури треба изоставити наслове рада.

8. Обавештавање аутора

8.1. Када рукопис буде прихваћен, аутор ће о приближном времену објављивања бити обавештен у писаној форми.

8.3. Исправљање текста припремљеног за штампу треба ограничити на штампарске грешке. Значајне промене текста ће се наплаћивати. Кориговани текст треба вратити уредништву у најкраћем могућем року.

8.3. Аутори добијају 50 бесплатних примерака сепарата. Ако аутор жели већи број сепарата може их наручити код издавача уз надокнаду.

9. КОПИЈА РАДА НА ДИСКЕТИ

После прихватања рада потребно је доставити дискету са коначном верзијом рада. Дискета треба да садржи текст рада, табеле и слике (прилоге) на DD или HD дискети од 3,5 инча. Приложите и једну копију одштампаног рада ради лакше обраде табела и слика. Молимо да обраду текста вршите програмом Word for Windows (било која верзија). Приликом копирања рада на дискету, придржавајте се следеће процедуре: File> Save as>Options>Embed True Type fonts>ok>Save.