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Microbial Ectoenzyme Activity: Useful Parameters for Characterizing the Trophic Conditions of Lakes

B. Kiersztyn, W. Siuda, R. J. Chróst

Department of Microbial Ecology, Institute of Microbiology,
University of Warsaw, Poland

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Abstract

This report describes the relationship between microbial enzymatic activities (aminopeptidase, esterase, and alkaline phosphatase) and the trophic state index of the studied lakes. Pelagic surface water samples were collected from nineteen lakes (Mazurian Lake District, northeastern Poland), characterized by different degrees of eutrophication, during spring homothermy and summer thermal stratification periods in 1999 and 2000. Aminopeptidase and esterase activities of microbial assemblages in unfiltered water samples were positively proportional to the trophic conditions of the studied lakes and both enzymes significantly correlated with Carlson's trophic state index of lakes. No correlation between alkaline phosphatase activities and the trophic state index of the studied lakes was found. This study showed that the selected parameters of microbial activity are very useful for the rapid determination of actual trophic conditions in lake ecosystems.

Keywords: tropic status, ectoenzymes, aminopeptidase, esterase, phosphatase

Introduction

Trophic classification of aquatic environments is necessary for the characterization of water resources that are potentially available for human activities. Eutrophication of aquatic environments, which is both the reason for and the result of their trophic status, depends on a great variety of different processes and factors. Therefore, the trophic concept is multidimensional and involves several aspects of lake productivity, nutrient loading and concentration, faunal and floral quality and quantity, and lake morphometry [1]. Indexes that are based on all these indices describe most precisely the trophic status of aquatic environments. However, the high complexity of aquatic environments causes even multiparameter trophic classification to be sometimes unequivocal and limited in application [2]. Depending on specific situations it might be possible to consider the same lake as oligotrophic by one set of criteria and eutrophic by another. Moreover, the number of parameters that must be measured is often too complicated and laborious for routine frequent monitoring of water resources.

The alternative to the classical, multidimensional concept of the trophic status of water environments are approaches that consider only single criterion. They were introduced by Rodhe [3] and Beeton & Edmondson [4] and developed by Carlson [1]. The trophic state index (TSI) proposed by Carlson and based on one of three possible estimators (Secchi disc water transparency, $chlorophyll_a$ and/or total phosphorus concentrations) has up to now been the most popular and commonly used approach for determining the trophic status of aquatic environments. According to Carlson [1] and Carlson & Simpson [5] none of the single parameters mentioned above may serve as a universal key for constructing a trophic classification of lakes. They ought to be used

Correspondence to: Prof. Ryszard J. Chrost

alternatively, depending on actual physico-chemical and biological conditions of a described lake. Moreover, it has been suggested that priority be given rather to biological parameters for their use as the trophic state indicator. All parameters proposed by Carlson for TSI estimation (i.e. water transparency, chlorophyll$_a$ and total phosphorus concentrations) are related, directly or non-directly, to phytoplankton biomass. It should be pointed out that until now there has been no approach that applies microbial parameters for TSI determination in aquatic ecosystems.

The main goal of our studies was to re-examine Carlson's classical trophic state index in respect to the "microbial" component of aquatic environments and to evaluate and discuss the relationship between ectoenzymatic activities of heterotrophic bacteria and the trophic state index of the studied lakes.

Methods

Study Area and Sampling

The studies were carried out during two vegetation periods (spring and summer 1999 and 2000) in nineteen lakes of the Mazurian Lake District in northeastern Poland. Table 1 presents the basic characteristics of the studied lakes: Przystan, Mamry, Dargin, Labap, Kisajno, Niegocin, Boczne, Jagodne, Szymoneckie, Szymon, Tattowisko, Rynskie, Talty, Mikolajskie, Beldany, Sniardwy, Kuc, Glebokie, and Majcz. These lakes represented different eutrophication conditions and varied from oligo/mesotrophic Lake Przystan to highly eutrophic Lake Szymon. Twice a year (in April, during water homothermy, and in July, during summer thermal stratification) ten subsamples (1 liter) of pelagic surface water (0 - 0.5 m depth) were taken from 10 sampling sites situated along the transect length of each lake. Subsamples were mixed vol/vol to obtain one integrated sample representative of each studied lake. Integrated samples were immediately transported to our laboratory in polyethylene 5 liters containers.

Physico-chemical Parameters

Chlorophylla (Chla) was extracted from phytoplankton with 98% ethanol and measured by spectrophotometry [6]. **Total phosphorus** (PT) was determined spectrophotometrically as orthophosphate after chemical hydrolysis and oxidation of organic P compounds in unfiltered water samples [7]. **Water transparency** (WT) was measured in situ by Secchi disc visibility.

Ectoenzyme Activity

Esterase Activity

Esterase activity (EsA) was assayed fluorometrically as an increase of fluorescein concentration in water samples incubated with fluorescein-diacetate (FDA),

Table 1. Basic morphological parameters and trophic status of the studied lakes.

Lake	Surface area (ha)	Average depth (m)	Maximum depth (m)	TSI Chla (July)	Trophic condition
Kuc	99	8.0	28.8	44.9	Mesotrophy
Mamry	2504	11.7	43.8	44.4	Mesotrophy
Majcz	45	3.2	16.5	37.8	Oligo/mesotrophy
Przystań	115	n.d.	22.8	40.2	Mesotrophy
Łabap	350	8.5	13.4	51.4	Eutrophy
Dargin	2680	10.6	37.6	57.3	Eutrophy
Kisajno	1896	8.4	25.0	54.0	Eutrophy
Głębokie	47	15.0	34.0	41.9	Mesotrophy
Śniardwy	11340	5.8	23.4	55.5	Eutrophy
Niegocin	2600	9.9	39.7	56.6	Eutrophy
Boczne	183	8.4	25.0	56.1	Eutrophy
Bełdany	941	10.0	46.0	60.8	Eutrophy
Tałty	1160	13.5	44.7	60.9	Eutrophy
Tałtowisko	327	14.0	39.5	69.0	Eutrophy/hypereutrophy
Ryńskie	671	13.5	50.8	65.4	Eutrophy
Mikołajskie	498	11.2	25.9	63.6	Eutrophy
Jagodne	420	8.7	37.4	65.5	Eutrophy
Szymoneckie	523	8.7	28.5	65.9	Eutrophy
Szymon	154	1.1	2.9	65.5	Eutrophy

a non-fluorescent esterase substrate [8]. To determinate the kinetics of FDA hydrolysis, a series of subsamples (3.8 ml) taken from each water sample was buffered with 0.1 ml of 0.5 M Tris-HCl buffer (pH = 7.2) and supplemented with 0.1 ml of appropriate substrate working solutions. Final concentrations of FDA in assay were: 0.5, 1.0, 2.5, 4.0, 5.0, 7.5, 10.0, 15.0, 17.5 µM. Substrate stock solution (2 mM FDA in acetone) and working solutions, obtained by dillution of FDA stock solution in pure acetone, were freshly prepared before EsA determinations. Fluorescence of samples was measured at 489 nm (excitation) and 510 nm (emission) in spectrofluorometer (Shimadzu RF 1501) at time zero, immediately after substrate addition (control), and after 0.5 -1.0 h of incubation.

Aminopeptidase Activity

Aminopeptidase activity (AMP) was measured fluorometrically [9] as rates of 7-amino-4-methyl-coumarin (AMC) released in the course of L-leucine--4-methyl-cumarinylamid hydrochloride (Leu-MCA) hydrolysis by the aminopeptidase present in water samples. For aminopeptidase assays, 0.1 ml of appropriate substrate solutions in deionized water were added to 3.9 ml of water samples, yielding final Leu-AMC concentration in: 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 µM. Fluorescence of AMC, at zero time and after 0.5 -1.0 h incubation, was determined spectrofluorometrically (380 nm excitation and 460 nm emission) in a Shimadzu RF 1501 spectrofluorometer.

Alkaline Phosphatase

Alkaline phosphatase (APA) activity was determined as the rates of increase in fluorescence of methylumbelliferone (MUF) (365 nm excitation and 460 nm emission) resulting from enzymatic hydrolysis of non-fluorescent substrate methylumbelliferyl-phosphate (MUFP) [10]. To determine the kinetic parameters of MUFP hydrolysis by APA two sets of water samples (3.8 ml) were prepared. Samples of the first set (blanks) were buffered (to inactivate APA and to yield maximum MUF fluorescence) to pH 10.9 with 0.1 ml of 1 M glycine-NaCl buffer and then supplemented with 0.1 ml appropriate substrate solutions (0.1, 0.25, 1.0, 2.5, 3.0, 5.0, 7.5, 10.0, 15.0 µM in assay). Their fluorescence was determined after 0.25 - 1.0 h incubation. The second set of samples (tested samples) was supplemented with the same amounts of substrate, and buffer was at the end of incubation (0.25 - 1.0 h). The increase of MUF fluorescence by APA hydrolysis was calculated as the difference between fluorescence of MUF in tested samples and fluorescence of the blanks. Fliorescence of samples was measured in spectrofluorometer Shimadzu RF 1501. Fluorescence was recalculated to concentration of MUF (which was equal to the concentration of enzymatically liberated PO_4^{3-}) with a standard curve.

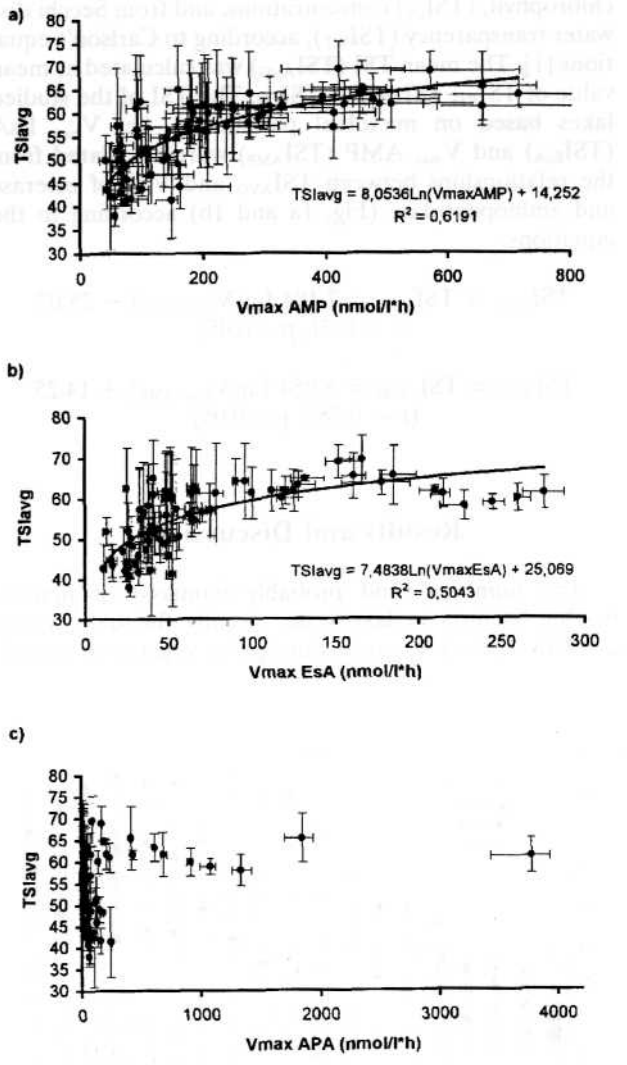

Fig. 1. Relationship between average TSI and a) V_{max} of aminopeptidase, b) V_{ma}, of esterase, and c) V_{max} of phosphatese in surface water of the lakes.

Calculations Analysis of

Enzyme Kinetics

Each tested enzyme-substrate system followed first order Michaelis-Menten's kinetics. Plot of the reaction velocity (v) against substrate concentration [S] always displayed a rectangular hyperbola relationship, according to the equation:

$$v = (V_{max} \times [S]) / (K_m + [S])$$

Nonlinear regression analysis was applied to calculate the kinetic parameters of enzymatic reactions by means of PC software Enzfitter (Biosoft, U.K.).

Trophic State Index (TSI) Calculations

The trophic state index for the studied lakes was independently calculated from total phosphorus (TSI_{PT}) and

chlorophyll$_a$ (TS_{chl}) concentrations, and from Secchi disc water transparency (TSI_{WT}), according to Carlson's equations [1]. The mean TSI (TSI_{AVG}) was calculated as mean value of TSI_{PT}, TSI_{WT} and TSI_{Chl}. The TSI of the studied lakes based on microbial parameters, i.e. V_{max} EsA (TSI_{ESA}) and V_{max} AMP (TSI_{AMP}) were calculated from the relationships between TSI_{AVG} and V_{max} of esterase and aminopeptidase (Fig. 1a and 1b) according to the equations:

$$TSI_{AVG} = TSI_{EsA} = 7.484 \, Ln(V_{max\ EsA}) + 25.07 \ (r = 0.71, p < 0.05)$$

$$TSI_{AVG} = TSI_{AMP} = 8.054 \, Ln(V_{max\ AMP}) + 14.25 \ (r = 0.787, p < 0.05)$$

Results and Discussion

The numbers (and probably biomass) of heterotrophic bacteria in lake water usually fluctuate within a relatively narrow range during the vegetation period.

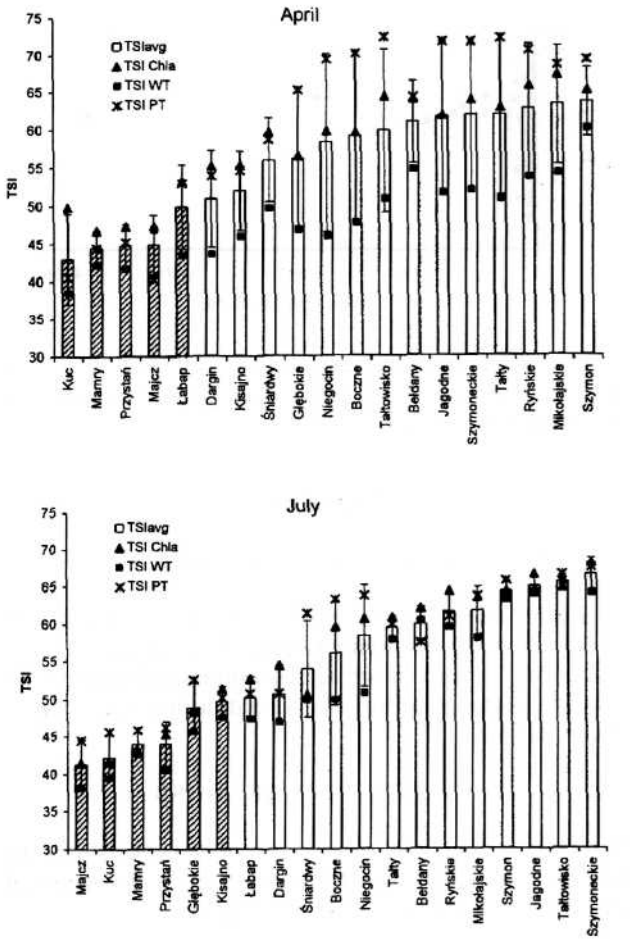

Fig. 2. Values of TSI_{WT}, TSI_{chla}, TSI_{PT} and average TSI calculated for a) April and b) July for all tested lakes. Lakes qualified as weakly eutrophic - stripped bars and highly eutrophic - open bars.

This changes generally within one order of magnitude. According to Koton-Czarnecka & Chrost [11] up to about 50% of bacterial production can be consumed by protozooplankton within the microbial loop ("top down control"). Growth and activity of bacterial populations are also controlled by the availability of organic substrates in aquatic environments ("bottom up control"), i.e. eutrophication conditions of the environment and/or its trophic status. In our studies we have tested the assumption that selected microbial activities (ectoenzymes) are be tightly coupled to the trophic state index of the studied lakes.

We calculated Carlson's trophic state indexes for all studied lakes from Secchi disc water transparency, and total phosphorus and chlorophyll$_a$ concentrations. Values of TSI_{WT}, TSI_{TP} and TSIchi calculated separately for April and July are presented in Fig. 2. TSI values estimated for the same lake varied notably during sampling periods, as well as their values depended on parameter used for their calculation. Carlson's TSI calculated from Secchi disc visibility revealed TSIWT values that did not change significantly between different study periods. Generally (except in a few less eutrophicated lakes examined in spring) TSI values calculated from water transparency were about 20-30% lower than those calculated from total phosphorus. To narrow the range of differences between the trophic state indexes calculated from various parameters we calculated the mean TSI values [$(TSI_{WT} + TSI_{TP} + TSI_{chla})/3$] and used them in further analyses.

Most evident differences in mean TSI values during both seasons were observed in the case of lakes qualified as less (open bars) and highly (stripped bars) eutrophic (Fig. 2). None of the 19 studied lakes were qualified, with regard to their mean TSI, in the same order in spring and in summer.

Linear regression analysis was applied to examine the relationships between trophic state indexes and microbial ectoenzymatic activities (aminopeptidase, alkaline phosphatase and esterase) in the studied lakes. Studied emzymes are commonly produced by a variety of aquatic microorganisms, and their activities in lake water often predominate among other enzymes. We found that maximal potential activities (V_{max}) of aminopeptidase (Fig. 1A) and esterase (Fig. IB) positively correlated with mean TSI values. However, we observed no relationship between mean TSI and alkaline phosphatase activities (Fig. 1C).

Correlation between aminopeptidase activity and bacterial biomass was reported in earlier studies [9,12]. Proteins (aminopeptidase substrates) are one of the most common nitrogen rich constituents in DOM fraction [13, 14], and they are the most important, easily utilizable nitrogen, carbon and energy sources for aquatic microheterotrophs. Aminopeptidase activity is produced constitutively by bacteria [12]. Therefore, its activity is tightly related to bacterial number and to some extent is proportional to bacterial biomass. Similarly, changes in the overall activity of microbial esterase reflect probably both the standing crop of heterotrophic bacteria and amount of various DOM constituents rich with ester bonds.

The activity of alkaline phosphatase was suggested as a useful determinant of the trophic status of aquatic environments [15]. However, results of our studies (Fig. 1C) did not confirm this assumption. From the theoretical

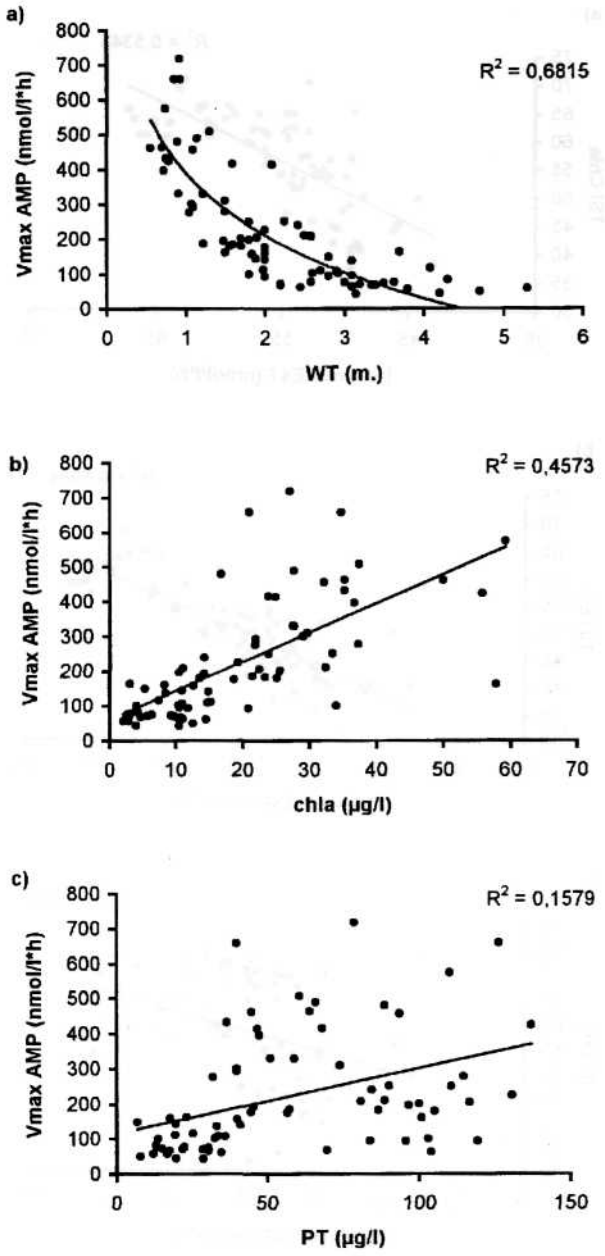

Fig. 3. Relationship between AMP activity and a) Secchi disc visibility, b) chlorophill a concentration c) Total phosphorus concentration in studied lakes.

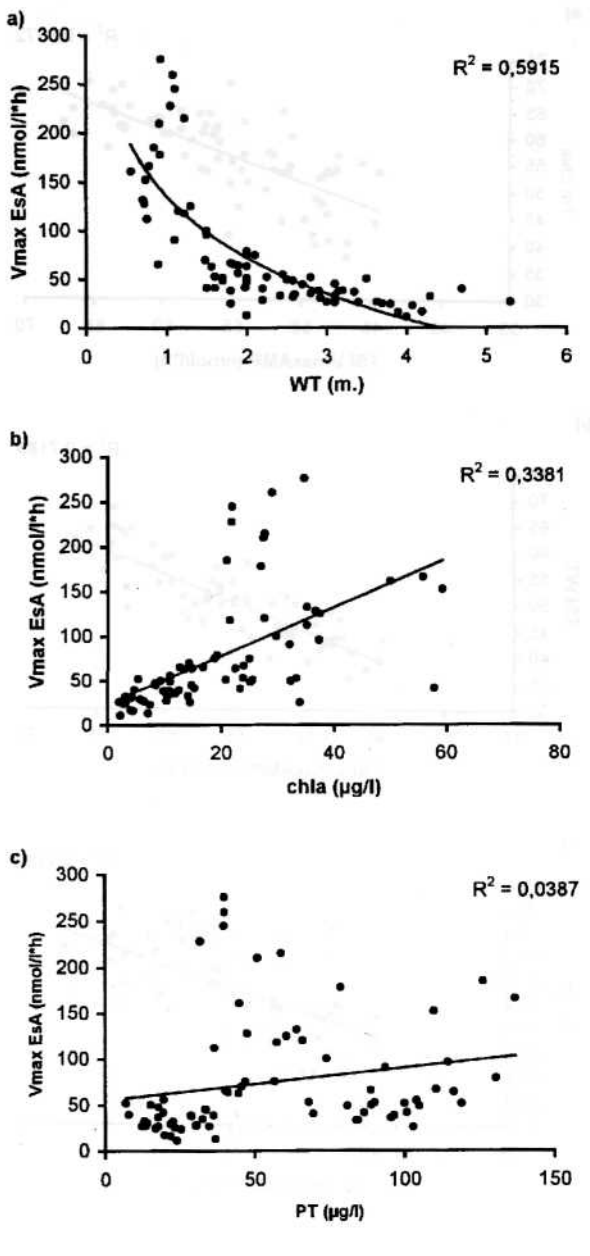

Fig. 4. Relationship between EsA activity and a) Secchi disc visibility, b) chlorophill a concentration c) Total phosphorus concentration in studied lakes.

point of view there is no reason to expect a correlation between microplankton biomass and activity of this adaptive enzyme that is synthesized by both autotrophic and heterotrophic microorganisms. We think that one of the possible explanations of this discrepancy between results of Jones's [15] studies and ours may be the use of different methods for APA determination. The extremely sensitive fluorometric method applied in our studies may quantify a different aspect of activity of APA assemblages than the old, much less sensitive colorimetric method used by Jones [15]. Another possible explanation is that APA may also have "two step" kinetics as described in the case of DNA-se [16].

The high and statistically significant correlation of AMP and EsA activities with TSI of the studied lakes (Fig. 1) and with parameters used for TSI estimation (water transparency and chlorophyll$_a$ concentration) (Figs. 3 A,B and 4 A,B) suggest that activity of these ectoenzymes may be useful microbial indices for evaluating of the trophic state index of aquatic environments. The results of more detailed analysis of suitability of these microbial parameters for trophic classification of lakes are presented in Figs. 5 and 6. Generally, both indexes calculated from AMP and EsA activities closely correlate with Carlson's TSI_{WT}, TSI_{Chla} and TSI_{TP} The highest and statistically most significant correlation was between $TSIV_{max\ AMP}$ and TSI_{WT}, and $TSI_{Vmax\ EsA}$ and TSI_{WT} ($r^2 = 0.72$, $p < 0.005$, and $r^2 = 0.70$, $p < 0.005$,

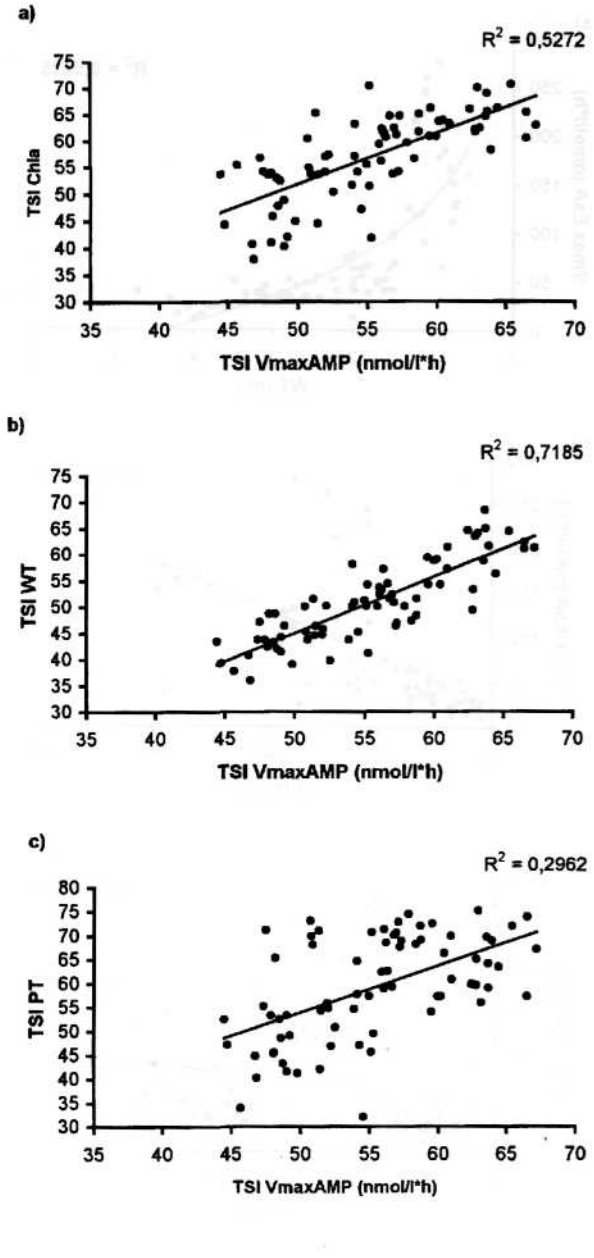

Fig. 5. Relationship between TSI V_{maxAMP} and a) TSI_{Chla} b) TSI_{WT}, c) TSI_{PT} calculated for studied lakes.

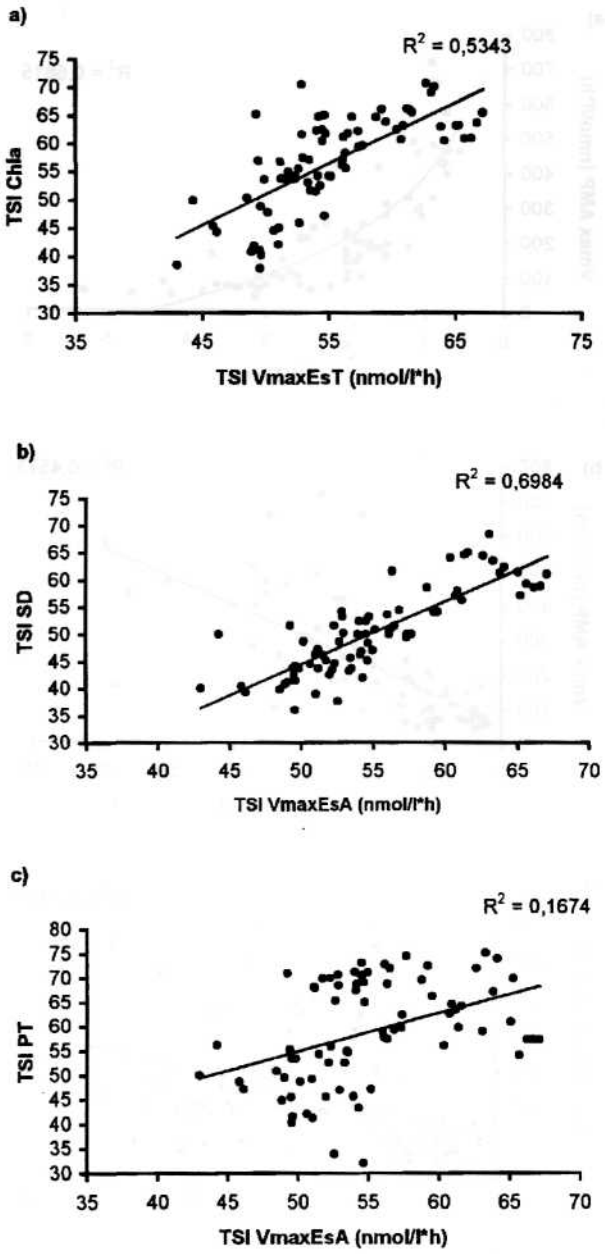

Fig. 6. Relationship between TSI V_{maxEsA} and a) TSI_{Chla}, b) TSI_{WT}, c) TSI_{PT} calculated for studied lakes.

respectively). Distinct correlations were also found in the case of TSI_{Chl} and $TSI_{Vmax\ AMP}$ ($r^2 = 0.53$, $p < 0.005$), and $TSIV_{max\ ESA}$ and TSI_{Chl} ($r^2 = 0.53$, $p < 0.005$). The lowest but still statistically significant correlation was observed between both "enzymatic" TSI and TSI_{PT} ($TSI_{Vmax\ AMP}$, $r^2 = 0.30$, $p < 0.05$ and and $TSI_{Vmax\ EsA}$, $r^2 = 0.17$, $p < 0.05$, respectively).

Our observations show that the "enzymatic" TSI approach is useful for characterizing the trophic conditions of aquatic environments. "Enzymatic" TSI is closely related to the average value of all classical indexes and thus is more universal. TSI indexes based on enzymatic activities describe better than other methods the trophic status of lake during periodically occurring algal blooms and allow for more precise differentiation between highly eutrophicated environments. Both enzymatic TSI indexes also permit us to characterize trophic conditions of polyhumic (brown waters) lakes and turbid environments, where Secchi disc water transparency cannot be used for Carlson's TSI determination. Moreover, the enzymatic trophic state indexes proposed in this study may be very useful for describing both microbial activity and the current trophic conditions of aquatic environments.

Acknowledgments

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