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Study of the impact of TIN/PO₄ ratio on mucilage formation in the northern Adriatic using regression trees

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The north-western part of the northern Adriatic (NA) exhibits eutrophic to mesotrophic characteristics with recurrent algal blooms and quite unpredictable mucilage events. To contribute to the understanding of the mucilage events in the NA a machine learning algorithm for induction of regression trees was applied on a long-term data-set comprising physical, chemical and biological parameters, measured at six stations on the profile from the Po River delta (Italy) to Rovinj (Croatia). A model describing the connection between the TIN/PO₄ ratio, considered as a necessary factor and sometimes even a trigger for mucilage events, and the environmental conditions in NA, was elaborated. The model for TIN/PO₄ ratio confirmed the assumption that the mucilage events are connected with this ratio, e.g. mucilage events coincides with its high values. This finding indicates that at certain levels of phosphorus limitation (from TIN/PO₄ ratio) mucilage event frequency increases. The model also reveals that salinity and temperature are responsible for the changes of the TIN/PO₄ ratio and gives an insight on their threshold values which lead to high values of this ratio, further related to mucilage events.

Key words: TIN/PO₄ ratio, mucilage events, machine learning, regression trees, northern Adriatic

INTRODUCTION

Massive mucilage events are characterized by formation of aggregates of various shapes and dimensions (few millimetres up to several meters) in upper water column and kilometre long gelatinous surface fronts which occur mainly during summer with various durations (1-3 months). As hypothesised by HERNDL &

PEDUZZI, (1988); HERNDL, (1992) and FOGG, (1995), mucilage aggregates are an exacerbated and evolving stage of the marine snow appearing in environmental conditions able to favour the progressive coalescence of marine aggregates. Mucilage events have been documented several times during the past three centuries in the NA, first observations dating back in 1729 (FONDA UMANI *et al.*, 1989). While they were quite seldom

in the past, approximately once per 10-50 years, their frequency significantly increased since 1988 (RUSSO *et al.*, 2005), i.e., mucilage events were observed last time in 1988, 1989, 1991, 1997, 2000-2004 and 2007 (STACHOWITSCH *et al.*, 1990; DEGOBBIS *et al.*, 1995, 1999; VOLLENWEIDER *et al.*, 1995; COZZI *et al.*, 2004; PRECALI *et al.*, 2005; DELAZZARI *et al.*, 2008; CMR, unpub. data). Notably, only during the 1988, 1989 and 2004 events, when winds blowing from the sea to the land dominated, the coast has been contaminated by gelatinous material for weeks. Mucilage events have been also recorded in other parts of the Mediterranean, i.e. in the Central and Southern parts of the Adriatic Sea (DEGOBBIS *et al.*, 1995; GIANI & CORNELLO, 2004; PRECALI *et al.*, 2005), in the Tyrrhenian Sea (INNAMORATI, 1995; INNAMORATI *et al.*, 2001) as well as in the Eastern Mediterranean (GOTSIS-SKRETAS, 1995; SCULLOS *et al.*, 2006).

The mechanisms of mucilage formation and release are rather complex and still not completely clear. A very generic hypothesis is that mucilage formations are produced by algae in stressed environmental conditions. DESERTI *et al.* (2005) reveal the connections between the mucilage and the change of the climatic conditions using classical statistical analysis. In their research they compared seasonal anomalies of temperature (calculated as spatial mean over the Po Valley area), and anomalies of North Atlantic Oscillation (NAO) with the historical record of mucilage episodes. Both climatic indices were found to be positively correlated with mucilage events, suggesting a possible relationship between climatic variability and the increased appearance of mucilage aggregates. The descriptive analysis pointed out that the mucilage events could be grouped in three main clusters: (1) 1920-1930, (2) 1983-1991 and (3) 1997-2002. TOTTI *et al.* (2005) used principal component analysis (PCA) to discover a relationship between phytoplankton size-distribution and community structure in relation to mucilage occurrence in NA, and they found that several phytoplankton species (diatoms and dinoflagellates) may contribute to the aggregate formation and enlargement, but mucilage

aggregates themselves may also affect the phytoplankton populations, allowing the development of a rich diatom community and in general enhancing nanophytoplankton growth.

There is a large consensus that the mucilage phenomenon is generated by synergetic effects of several factors (i.e. DEGOBBIS *et al.*, 1999). One of the most significant factors is the change of TIN/PO₄ ratio which can increase the phytoplankton excretion of polysaccharide mucus, the matrix of the mucilaginous material (i.e. MYKLESTAD, 1995; GRILLI *et al.*, 2005). COZZI *et al.* (2004), GRILLI *et al.* (2005), PENNA *et al.* (2009) and TURK *et al.* (2010) indicate that for the development of the phenomenon, large variations of the nutrient fluxes and their ratios, in conditions of a marked retention of freshened waters in the NA and water column stratification, are more important than the absolute amounts of the nutrient inputs. DEGOBBIS *et al.* (2005) investigated the changes in the nutrient concentrations and ratios during mucilage events and showed the important role of TIN/PO₄ ratio in the formation of mucilage events using statistical and graphical analysis. Also, they revealed that the orthosilicate (SiO₄) did not play a significant limiting role in phytoplankton growth so it is probably not essential for the development of the mucilage phenomenon.

In the case of the NA the Po River, with an average flow rate of 1500 m³ s⁻¹ (period 1986-2001) is the most important source of nutrients with a load of around 170×10³ t a⁻¹ of total nitrogen and 8×10³ t a⁻¹ of total phosphorus for period 1996-2001 (PALMERI *et al.*, 2005; COZZI & GIANI, 2011). This nutrient enriched water can be transported over large areas of the NA and greatly influence the nutrient distribution and, consequently, the primary production of the surface layer (GILMARTIN *et al.*, 1990). The changes in nutrient ratios in the surface layer of the NA, influenced by the Po River discharges, coincided with an increased frequency of mucilage events (GRILLI *et al.*, 2005). However, nutrient concentrations and their ratios vary greatly each year in the NA, but not necessarily cause mucilage events. This indicates that more factors, not just TIN/PO₄ ratio change are involved in the

triggering mechanisms of these phenomena. The nutrients role in the development of the mucilage phenomenon, suggested by laboratory and controlled by *in situ* experiments, could become critical within particular circulation patterns and conditions (GRILLI *et al.*, 2005).

In this work we advance the present understanding of the nutrients' ratio influence to the mucilage development by providing a descriptive model for more detailed insight into the process and revealing specific values of the TIN/PO₄ ratio changes that can trigger the mucilage events. Moreover we model the environmental conditions that enable those ratio values.

For this purpose, a machine learning algorithm was used, i.e. an algorithm for induction of regression tree model from measured long term data in the NA (from 1972-2007). Unlike other data-driven methods applied previously to the NA data, i.e. classical (DEGOBBIS *et al.*, 2005, DESERTI *et al.*, 2005, GRILLI *et al.*, 2005) and advanced statistical approaches, such as principal component analysis (PCA; BERNARDI AUBRY *et al.*, 2004; TOTTI *et al.*, 2005, TEDESCO *et al.*, 2007, FANUKO *et al.*, 2008) or self-organizing map (SOM; SOLIDORO *et al.*, 2009), which provide very useful insights in the data, but sometimes limited in terms of interpretability, regression trees tend to be more descriptive and interpretable (KOM-PARE, 1995; KOMPARE *et al.*, 2001; ATANASOVA *et al.*, 2008; DZEROSKI, 2009; VOLF *et al.*, 2011). Regarding the models accuracy they can successfully compete with e.g. neural networks, known for their accurate predictions (SOLOMATINE & DULAL, 2003). First attempt of applying regression trees to NA data set for predicting mucilage events did not use a complete data set and thus failed to predict all mucilage events in the observed period (VOLF *et al.*, 2013)

In this study we apply regression-based machine learning (ML) method on comprehensive data set in NA. The objective is to build a reliable model for TIN/PO₄ ratio and confirm it as one of the major indicators for appearance of mucilage events, as well as indicate which environmental variables are responsible for the changes of the TIN/PO₄ ratio (and consequently for mucilage events).

The paper is organised as follows: In Section 2 we describe the measured data. Section 3 describes the modelling methods used in this paper while, Section 4 describes the experimental setup for the modelling tasks. Section 5 gives the results, i.e. the constructed models, in Section 6 is given discussion of the results and finally Section 7 contains the conclusions of this paper.

MATERIAL AND METHODS

Study area and sampling

The data set used for modelling and interpretation comprised physical, chemical and biological parameters. Data were collected at six stations (SJ108, SJ101, SJ103, SJ105, SJ107 and RV001) on the profile from 12 Nm off the Po River delta to 1 Nm off Rovinj on the western Istrian coast by the Center for Marine Research (CMR) in Rovinj, Croatia (Fig. 1). This transect is 92 km in length, with maximum station depth up to 37 m and is considered representative for the shallowest part of the NA delimited by the line Cape Kamenjak-Rimini, (REVELANTE & GILMARTIN, 1983; DEGOBBIS *et al.*, 2000), approximately down to the 50 m isobaths with a surface area of about 19 000 km² and a volume of 635 km³ (DEGOBBIS & GILMARTIN, 1990). Marked eutrophication gradients are often established between the predominantly mesotrophic north-western part of this region and its south-eastern part which is under the influence of oligotrophic waters originated in the central Adriatic.

The water column was sampled with 5 L Niskin samplers at 0.3, 5, 10, and 20 meters, and at 2 meters above the bottom from 1972-2007 with near monthly frequency.

Data analysis and sources

Nutrients analyses (ammonium-NH₄, nitrite-NO₂, nitrate-NO₃ and orthophosphate-PO₄) were performed on unfiltered samples aboard the research vessel immediately after sample collection. The analyses were performed by methods used in oceanographic research defined

by STRICKLAND & PARSONS (1972), using Beckman DU and Shimadzu UV mini-1240 and UV-1800 spectrophotometers with 10 cm cells. Method accuracies for NO_3 , NO_2 , NH_4 and PO_4 are 73 %, 73 %, 75 % and 73 %, respectively, and detection limits are 0.05, 0.01, 0.1 and 0.02 $\mu\text{mol L}^{-1}$, respectively. Temperature was measured with reversing thermometers, salinity by Beckman RS 7c or Yeo-Kal MKII high precision salinometers in the ashore laboratory. The samples for total phytoplankton counts (micro and nano fractions) were preserved with lugol solution and counted according to UTERMÖHL (1958) using Carl Zeiss inverse microscopes. Total inorganic nitrogen (TIN) was calculated as the sum of NH_4 , NO_2 , and NO_3 . Data analysis was made by the Center for Marine Research (CMR) in Rovinj, which also provide the data for this research.

Daily Po River flow data measured at Pontelagoscuro, 90 km from the outlet (Fig. 1) from January 1966 to December 2007 were obtained from the Agenzia Regionale Prevenzione e Ambiente dell'Emilia Romagna, Servizio Idrometeorologico, Parma. The data used for building the model are depicted in Table 1.

For the modelling experiments the entire span of the historic data was used. At each station the measured parameters for the top 10 m of the water column were averaged (mucilage phenomenon breaks out primarily in the upper water column). Additionally, we have information about the temporal occurrence of the

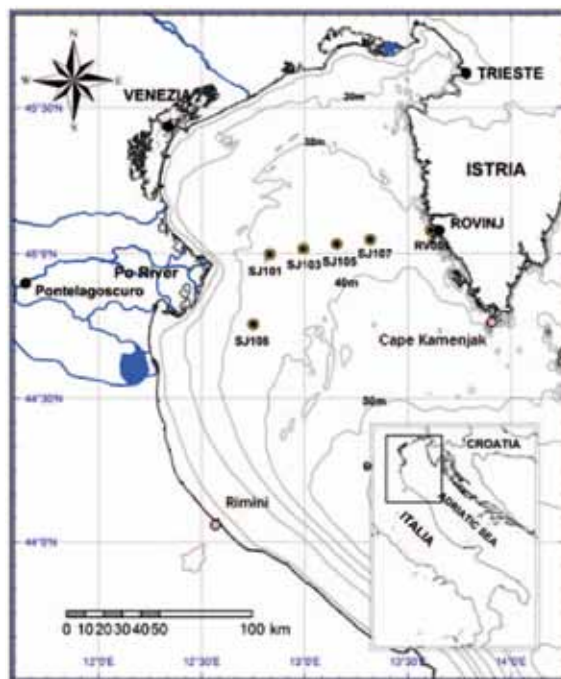


Fig. 1. Map of survey stations

mucilage events (CMR, Rovinj, unpub. data; see Figs. 7, 8 and 9).

Modelling method: regression trees

For a data set of examples in a form $(a_1, a_2, \dots, a_n, x)$, where a_i are values of the n attributes also called independent variables or descriptors and x is the value of the dependent variable (also called a target variable, or class), regression trees can be induced from the data to predict

Table 1. Parameters used for modelling and interpretation TIN/PO4

Parameter	Description	Unit
Flow	Po River flow	$\text{m}^3 \text{s}^{-1}$
Year	Year of sampling	
Month	Month of sampling	
Temp	Sea temperature	$^{\circ}\text{C}$
SAL	Salinity	
$\text{PO}_4\text{-P}$	Orthophosphate as P	$\mu\text{mol L}^{-1}$
$\text{NO}_3\text{-N}$	Nitrate as N	$\mu\text{mol L}^{-1}$
$\text{NO}_2\text{-N}$	Nitrite as N	$\mu\text{mol L}^{-1}$
$\text{NH}_4\text{-N}$	Ammonium as N	$\mu\text{mol L}^{-1}$
TIN*	Total Inorganic Nitrogen	$\mu\text{mol L}^{-1}$
Phyto	Total Phytoplankton	cells L^{-1}
TIN/PO₄ (target var. 1)	Tot. In. Nitrogen/Orthophosphate as P	mol/mol

* $\text{TIN} = \text{NH}_4\text{-N} + \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$

the value of the class based on linear regression principle. The task of the simple linear regression is to express the class value in form of:

$$x = a_1 * w_1 + a_2 * w_2 + \dots + a_n * w_n = \sum a_i * w_i$$

where w_i are weights, which are learned (calculated) from the *training set*.

Unlike the simple linear regression, which calculates one equation (one weight vector) for the entire data set, *piecewise or tree-structured regression* divides the data set to several subsets on which *uniform class value or linear equation* can be applied. The division to subsets is based on tests of the values of the input attributes, which are put as nodes in a regression tree. Thus, regression trees are hierarchical structures composed of nodes and branches, where the internal nodes contain tests on the input attributes. Each branch of an internal test corresponds to an outcome of the test, and the predictions for the values of the target variable (the class) are stored in the leaves, which are the terminal nodes in the tree. Leafs in regression trees contains a single average value for the class prediction. Fig. 2 illustrates the procedure of constructing regression trees.

One of the mostly used algorithm for induction of regression trees is the M5 algorithm (QUINLAN, 1992), based on the TDIDT (top-down induction of decision trees) algorithm (QUINLAN,

1986). For our experiments a variation of the M5 algorithm was used, called M5P implemented in the software package WEKA (WITTEN & FRANK, 2000).

After the tree is constructed from the training (learning) set of data, it is necessary to assess the model quality, i.e., the accuracy of prediction. This can be done by simulating the model on a testing set of data and comparing the predicted values of the target with the actual values. Another option is to employ cross-validation. The given (training) data set is partitioned on a chosen number of folds (n). In turn, each fold is used for testing, while the remainder (n-1 folds) is used for training. The final error is the averaged error of the all models throughout the procedure.

The size of the error between the actual and the predicted values can be calculated by several measures to evaluate the model accuracy: root mean-squared error, mean absolute error, root relative squared error, relative absolute error and correlation coefficient (R).

Modelling experiment

For the experiment the machine learning algorithm M5P for induction of regression trees, integrated in the WEKA modelling software (HALL *et al.*, 2009) was employed. The experiment was designed to elaborate a model for

DATA SET (EXAMPLES)

AT1	AT2	AT3	...	TARGET (CLASS)
3.67	8.500	0.005	...	2.133
4.15	7.207	0.005	...	2.601
5.32	8.357	0.011	...	3.718
7.80	7.929	0.005	...	3.481
8.11	7.096	0.005	...	1.791
9.36	7.804	0.005	...	1.128
10.87	6.018	0.005	...	1.471
11.10	7.400	0.006	...	1.521
10.23	5.457	0.011	...	0.869
8.39	5.486	0.014	...	0.535
7.42	5.486	0.013	...	1.034
4.06	8.307	0.005	...	1.636
...

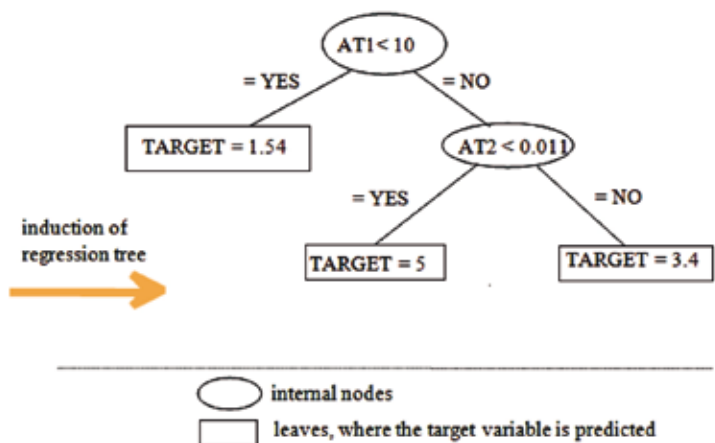


Fig. 2. Induction of regression trees from data set (examples)

TIN/PO₄ ratio. The ratio TIN/PO₄ was set as dependant variable, while Po River flow rate, year, month, sea water temperature, salinity and total phytoplankton were given as independent variables (see Table 1).

RESULTS

The goal of the model is to give an insight in how TIN/PO₄ ratio is changing in the NA marine ecosystem, and what are the most influential factors for this change, as this ratio is recognized as one of the most important and necessary factors if not a trigger for the mucilage production (HERNDL, 1992; DEGOBBIS *et al.*, 1999).

From the historical data from six stations (Table 1, Fig. 1) a TIN/PO₄ ratio model presented in Fig. 3, was constructed for the whole NA area. Parts A, B and C of the model tree on

Fig. 3 are shown as sub-trees on Fig. 4, 5 and 6. The accuracy of the model is given by the correlation coefficient (R) between the modelled and measured values of the TIN/PO₄ ratio. The correlation coefficient for the selected model (Fig. 3) using cross-validation method is 0.55 with probability of $p < 0.0001$.

The regression tree model in Fig. 3 shows the different average values of the TIN/PO₄ ratio for given time periods and under different conditions. The model is read in terms of IF-THEN rules, starting from the top node, e.g. *IF (Year ≤ 1999 and Temp > 19.6 and Flow ≤ 1015 and Year > 1995) THEN (TIN/PO₄ = 18.2)*. Analysing the tree, two characteristic periods for the value of the TIN/PO₄ ratio can be distinguished, where the environmental factors differently influenced the value of the ratio. These periods are before and after 1999 (see the top node of the

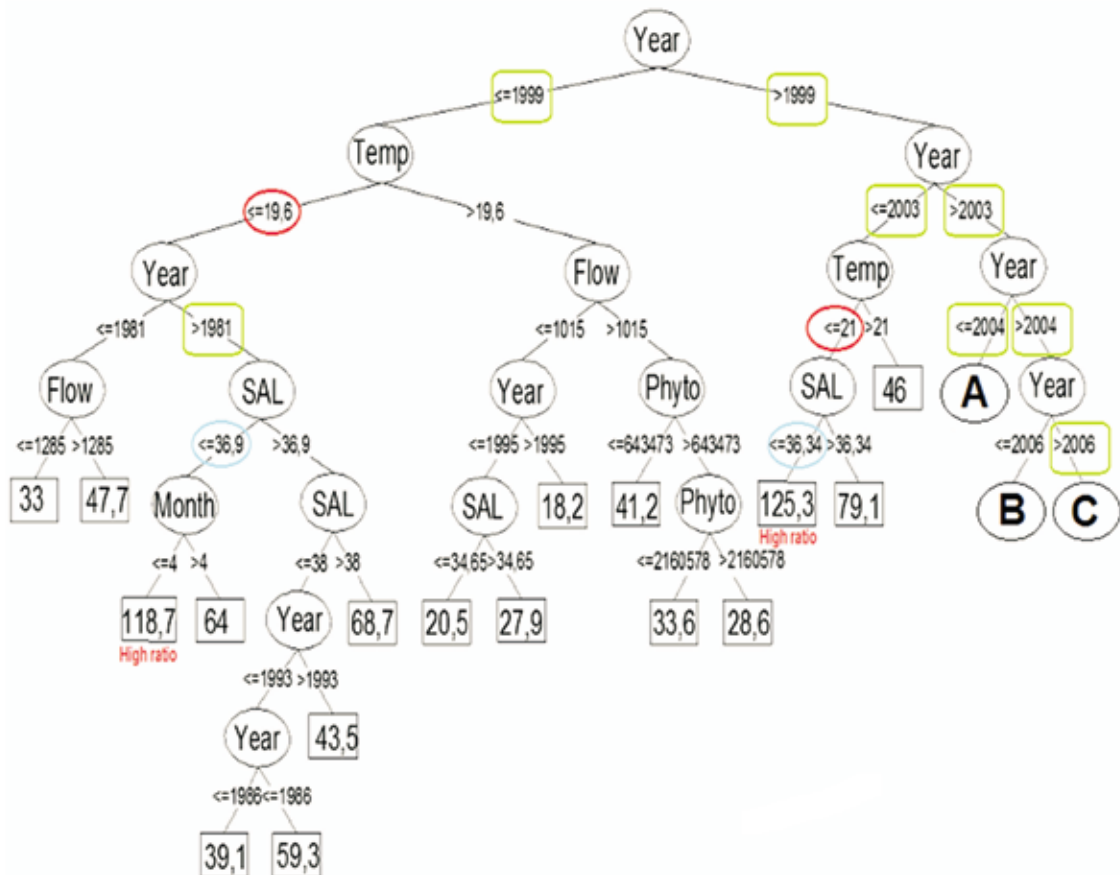


Fig. 3. Regression tree for TIN/PO₄ ratio. High values of the ratio coincide with observed mucilage events (units for the threshold values for the parameters used are reported in Table 1)

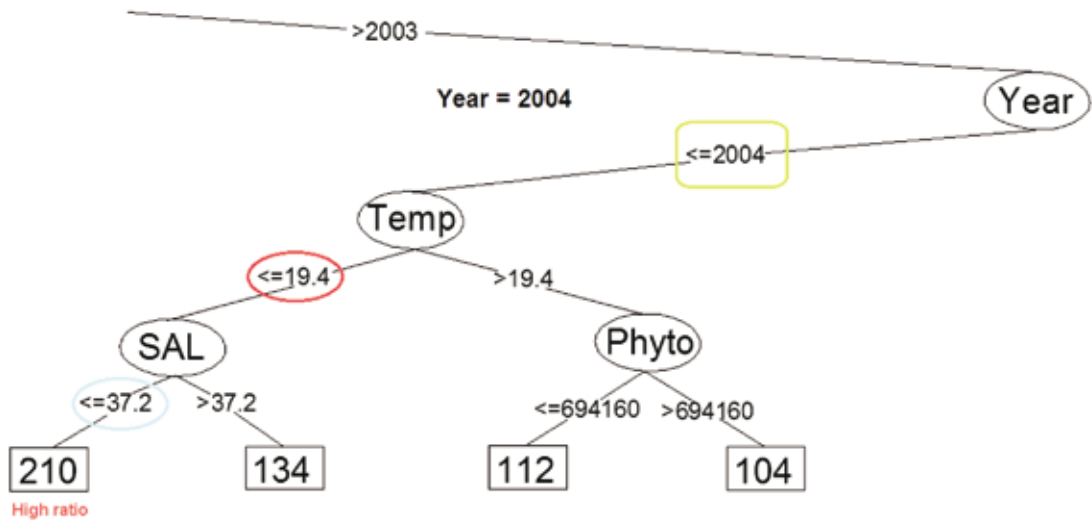


Fig. 4. Sub-tree (part A) for the regression tree presented in Figure 3 for year 2004

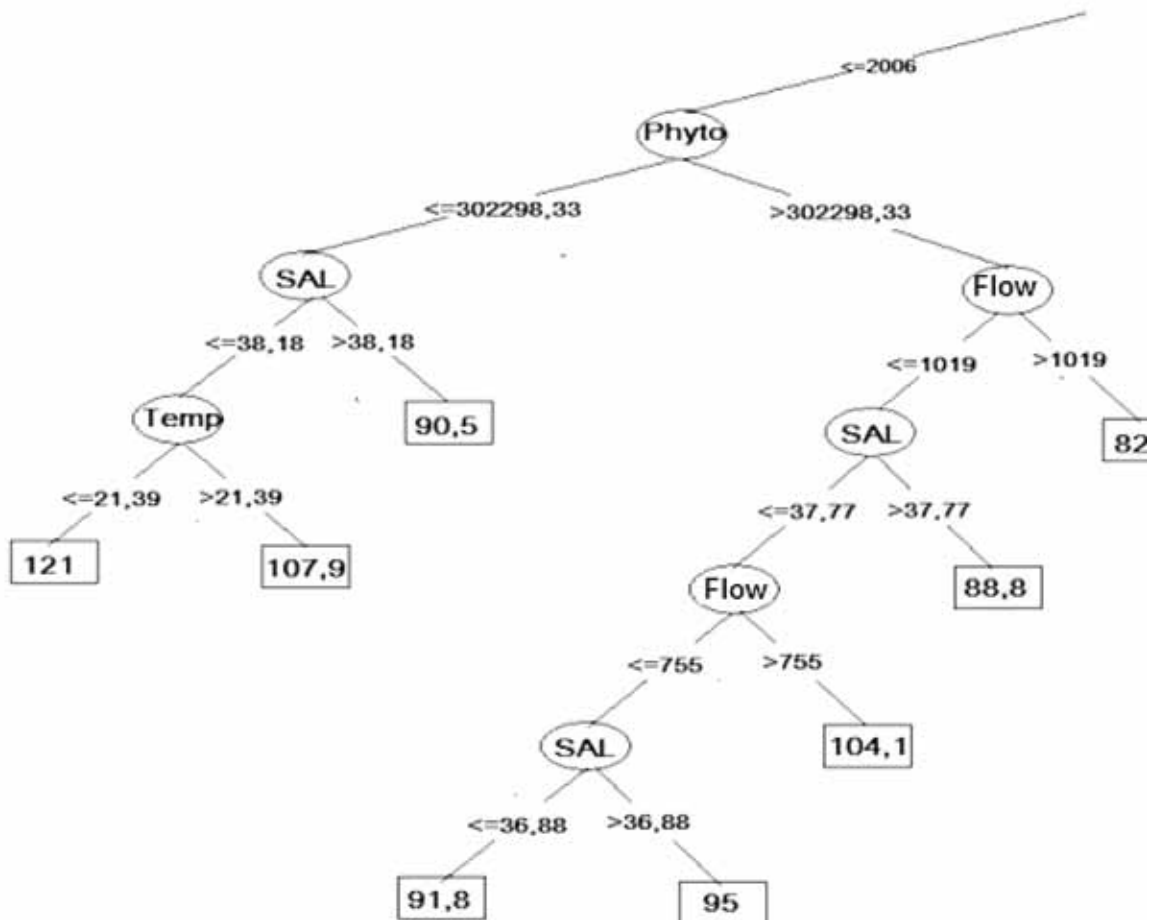


Fig. 5. Sub-tree (part B) for the regression tree presented in Figure 3 for years 2005 and 2006

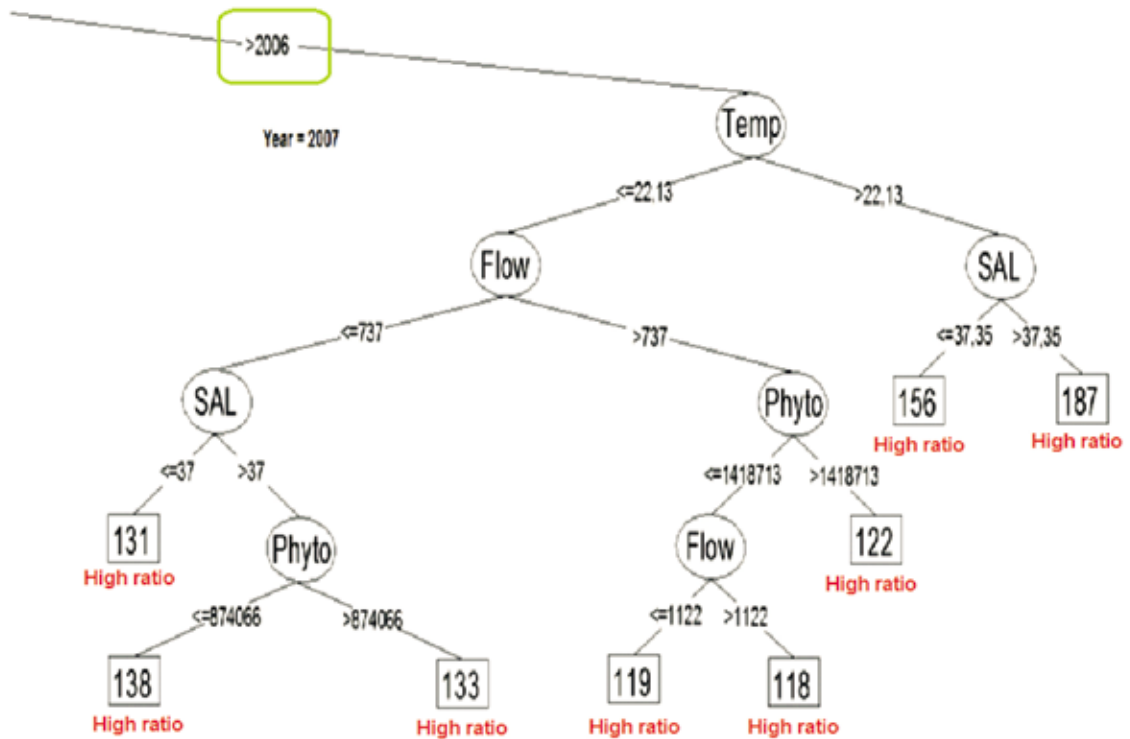


Fig. 6. Sub-tree (part C) for the regression tree presented in Figure 3 for year 2007.

tree in Fig. 3), i.e., periods from 1972-1999 and from 2000-2007.

In the first period the water temperature (Temp) was correlated with the ratio with a threshold value of 19.6 °C. Temperatures higher than 19.6 were related to relatively low values of the ratio (between 20.5 and 41.2). At lower temperatures different environmental influences were observed, i.e.:

1. In the period 1972-1981 only the Po River flow (Flow) influenced the value of the ratio. But, according to the model the average value of the ratio was always relatively low (33 and 47.7). No mucilage events were observed during this period. The rules that confirm this are read as follows:

1. IF (Year \leq 1999 and Temp \leq 19.6 and Year \leq 1981 and Flow \leq 1285) THEN (TIN/PO₄ = 33), and

2. IF (Year \leq 1999 and Temp \leq 19.6 and YEAR \leq 1981 and Flow $>$ 1285) THEN (TIN/PO₄ = 47.7)

2. In the period between 1982 and 1999 salinity (SAL) and month of the year (Month) influenced the average value of the ratio, with threshold values of 36.9 and 4 respectively. At lower salinity value (SAL \leq 36.9) and months before April (Month \leq 4) higher average TIN/PO₄ ratio value of 118.7 was observed, otherwise the average value was 64. Also, at higher salinity values (SAL $>$ 36.9) the average value of the ratio is between 39.1 and 68.7, much lower than the one of 118.7.

Interestingly, in this period mucilage events were observed during 1982-1999 i.e. in 1988, 1989, 1991 and 1997 and coincided with high average values of the TIN/PO₄ ratio (118.7). These values were triggered by temperatures lower than 19.6 °C, salinity lower than 36.9 and months of year before April.

In the second characteristic period (2000-2007), three main characteristic sub-periods can be distinguished (2000-2003, 2004 and 2005-2007). In the first (2000-2003) the model reveals high average TIN/PO₄ ratio value of 125.3 at

temperature lower than 21 °C and salinity lower than 36.34. Mucilage events appeared frequently in this period, i.e. in 2000, 2001, 2002 and in 2003. In the second sub-period (2004, Fig. 4) mucilage event is observed, where for high ratio value (TIN/PO₄ = 210) is responsible like in other periods temperature and salinity values lower than 19.4 °C and 37.2, respectively. Other values of ratio for this sub-period were between 104 and 134. In the third sub period (2005-2007, Fig. 5 and 6) the model also reveals high average TIN/PO₄ ratio values (range between 82.8-121) influenced by Phytoplankton. In 2005 and 2006 (Fig. 5) there were no mucilage events and when compared to 2007 (Fig. 6) when mucilage was observed again ratio values are not so high. Probably these high values are a result of a decrease of Po River inflow. For last 5 years a clear signal indicating a reduction of the available orthophosphate in the ecosystem was identified with an accumulation of inorganic nitrogen (MOZETIČ *et al.*, 2009). Year 2007 is characteristic because mucilage events were observed both in Spring-Summer and Autumn-Winter periods (CMR, unpub. data) with high ratio values (TIN/PO₄ = 118-187). Similarly to the first characteristic period (1972-1999), in the second one, mucilage events coincide with high TIN/PO₄ ratio values. However, compared to the first period, in the second period, sub-period 2000-2003, we observe different threshold values of temperature and salinity, i.e. 21 °C and 36.34, respectively. In the sub-period of 2004 the threshold values of temperature and salinity are similar to the period between 1982 and 1999 (19.6 and 19.4 °C, 36.9 and 37.2, respectively).

DISCUSSION

TIN/PO₄ ratio is considered to hide information on the possible trigger mechanisms for mucilage events. The model (Fig. 3) indicates when high average values of the TIN/PO₄ ratio occur mucilage events are possible (PENNA *et al.*, 2009, RICCI *et al.*, 2014). Recall that four mucilage events occurred between 1982 and 1999 (1988, 1989, 1991 and in 1997 when mucilage was observed, although less visible in the largest

part of the NA; WEB ADDRESS 1), four between 2000 and 2003 (2000, 2001, 2002 and 2003 - this event lasted less than one month), one in 2004 and one in 2007 (STACHOWITSCH *et al.*, 1990; DEGOBBIS *et al.*, 1995, 1999; VOLLENWEIDER *et al.*, 1995; COZZI *et al.*, 2004; PRECALI *et al.*, 2005; DELAZZARI *et al.*, 2008; CMR, unpub. data). This indicates that at certain levels of P limitation (the TIN/PO₄ signal clearly indicates this) mucilage events frequency increases.

The complete mechanism of mucilage formation could not be revealed in detail by this model due to insufficient amount of descriptors in the data set. Undoubtedly, the mucilage formation is a result of multiple factors, where the TIN/PO₄ ratio shows some of them. In this research mucilage events are identified indirectly, through the values of the TIN/PO₄ ratio solely. Given that the TIN/PO₄ ratio is one of the needed conditions for mucilage appearance (DEGOBBIS *et al.*, 2005), other conditions, not revealed in this study, need to be fulfilled for the mucilage appearance. The model is induced solely from measured data, and thus, if such information (pattern) does not exist in the training data it cannot appear in the model. Instead, the model indicates the favourable conditions for mucilage development, which could be revealed from the learning data set. The coincidence of the high TIN/PO₄ ratio with the mucilage events clearly indicates that the P limitation is one of the main triggering mechanisms. Still, from this ratio alone it cannot be reliably concluded if its effect on bacteria could affect the degradation of organic matter favouring accumulation of mucilage (i.e. AZAM *et al.*, 1999; PUGNETTI *et al.*, 2005). Namely, most important component in the mucilage organic matter is consisted of carbohydrates (RICCI *et al.*, 2014). In addition, not just TIN/PO₄, but also unbalanced C:N:P ratio can favour extensive mucus structures according to TURK *et al.* (2010).

Related to previous research of the mucilage phenomena in the NA, the model confirms some of the results, particularly those related to the effects of salinity and temperature on its formation, i.e. that the phenomenon is primarily developed at lower salinity (32-37) and oxygenated

surface waters (DEGOBBIS *et al.*, 2005). Recall the threshold values of salinity in the model (Fig. 3) of 36.9, 36.34, etc. PRECALI *et al.* (2005) showed that a major number of aggregates accumulated in correspondence with strong pycnoclines with differences in density anomaly of 2 kg m^{-3} or higher, due to temperature and salinity vertical changes. Observations of mucilage aggregate formations in 2000, 2001, and 2002 made by RUSSO *et al.* (2005) suggest that increased air and sea temperature could indicate the appearance of the mucilage phenomenon. Salinity as a measure of nutrient input, temperature and other factors influence the growth of planktonic algae in the NA, which can contribute to intense blooms in marine coastal waters (CUCCHIARI *et al.*, 2008). In the research done by RICCI *et al.* (2014) results indicated that variations in DIN/TP (Dissolved inorganic nitrogen/Total phosphorus) ratio, total dissolved carbohydrates, seawater temperature and salinity in spring months perhaps lead to the formation of mucilage. Finally, DESERTI *et al.* (2005) grouped the mucilage events (1920-2002) in three main clusters. While they identify two periods which are consistent with our dataset: 1983-1991 and 1997-2002, our model groups them into: 1982-1999, 2000-2003, 2004 and 2005-2007.

Additionally to the previous research, the model developed here, reveals the threshold values of salinity (SAL) and temperature (Temp) in the entire observed period (1972-2007) that lead to high values of the TIN/PO₄ ratio as indicator for mucilage events. Namely, the research made by PENNA *et al.* (2009) and RICCI *et al.* (2014) indicates that in spring before the appearance of macroaggregates, high concentrations of dissolved polysaccharides appeared in the waters of the NA, while the dissolved monosaccharide concentrations remained nearly constant. This was related to an increase of the DIN/PO₄ ratio in spring before the macroaggregate appearance, probably due to intense phytoplankton use of nitrate and phosphate, since during the years in which mucilage was not present this ratio was lower.

To show the model results in a more detail way, the mucilage events together with the environmental variables (temperature and salinity)

for the periods 1982-1999 (Fig. 7), 2000-2003 (Fig. 8) and 2004-2007 (Fig. 9) were graphically presented. Please note that this is not an exact representation, as the data were taken at different time scales. While temperature and salinity are measured monthly, the mucilage events are only observed in approximate time periods of their appearance (CMR, Rovinj, unpub. data). Still the results from the model in Fig. 3 are clearly confirmed. On each figure salinity (SAL), temperature (Temp) and TIN/PO₄ ratio values are presented together with their threshold values according to the model in Fig. 3.

Figures 7 and 8 indicate that the mucilage events occur when salinity (< 36.9 for 1982-1999 and < 36.34 for 2000-2003) and temperature ($< 19.6 \text{ }^\circ\text{C}$ for 1982-1999 and $< 21 \text{ }^\circ\text{C}$ for 2000-2003) are below the marked thresholds and above the predicted average value of TIN/PO₄ ratio with the model (above 118.7 for period 1982-1999 and 125.3 for 2000-2003).

Fig. 9 indicates that mucilage in 2004 when salinity and temperature were less than 37.2 and $19.4 \text{ }^\circ\text{C}$ respectively, are below the marked thresholds and above the predicted average value of TIN/PO₄ ratio with the model (above 210, Fig. 4). As said before, mucilage event in 2007 is specific because mucilage events were observed bigger part of the year (see Fig. 9). While the mechanism that leads to the mucilage events in Autumn-Winter periods is still not clear, in 2007 the TIN/PO₄ ratio values for the whole period were high (118-187), a rule confirmed by the model.

Figs. 7, 8 and 9 also reveal two important phenomena, which can not be revealed by the model in Fig. 3, for mucilage occurrence, i.e., 1) the mucilage events occur when sea-water temperature is increasing (see also DEGOBBIS *et al.*, 2005; RUSSO *et al.*, 2005), and 2) an obvious increase of phytoplankton concentration (Phyto) before the mucilage events (see also TOTTI *et al.*, 2005).

The temperature increase during a mucilage event cannot be confirmed by the model, as it only reveals the threshold values below which mucilage occur.

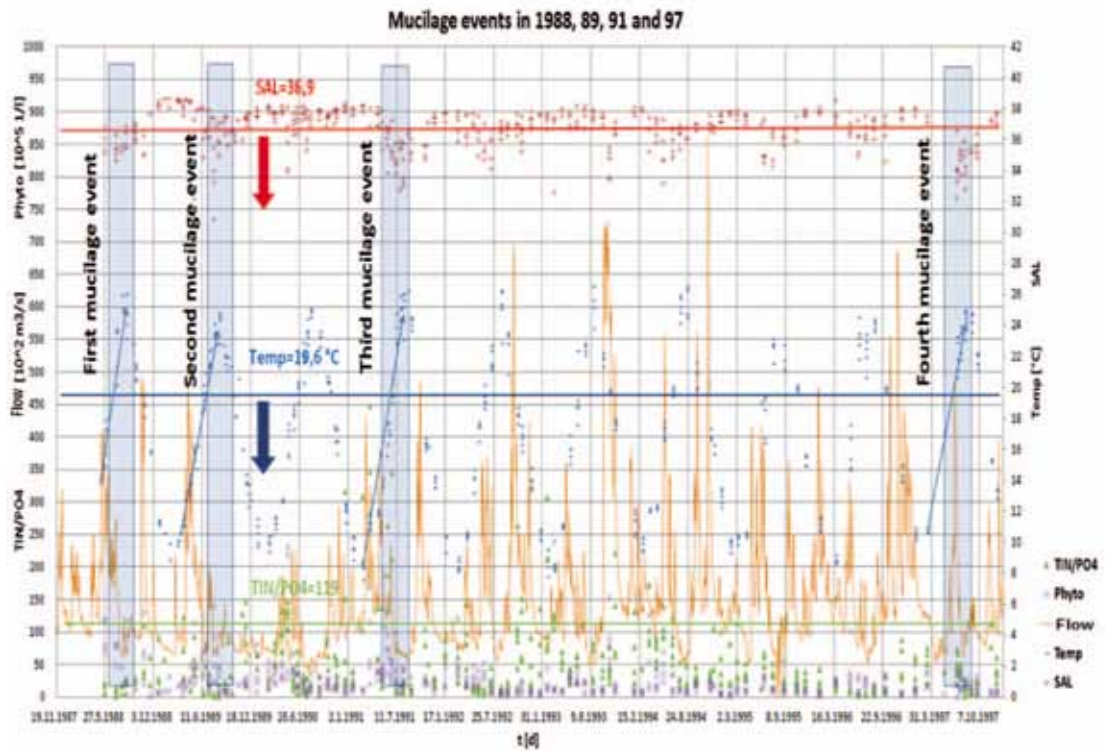


Fig. 7. Graphical presentation of TIN/PO₄ ratio, salinity, temperature, Po River flow and total phytoplankton with temporal occurrence of mucilage events in period 1982-1999

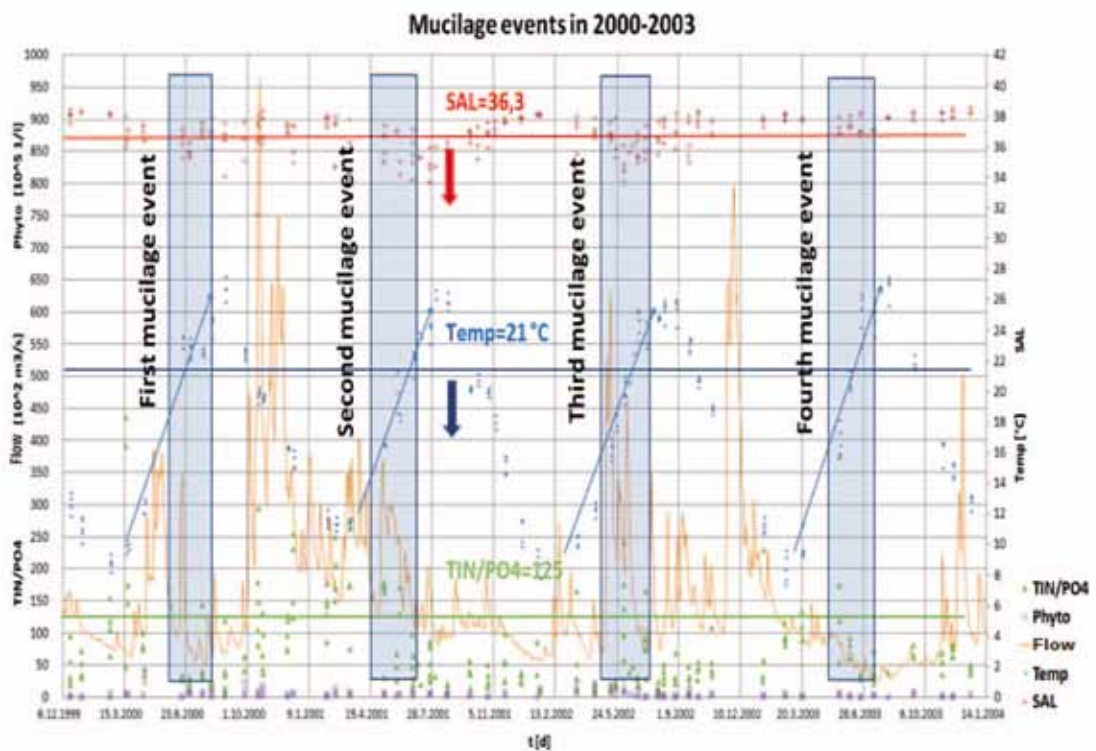


Fig. 8. Graphical presentation of TIN/PO₄ ratio, salinity, temperature, Po River flow and total phytoplankton with temporal occurrence of mucilage events in period 2000-2003

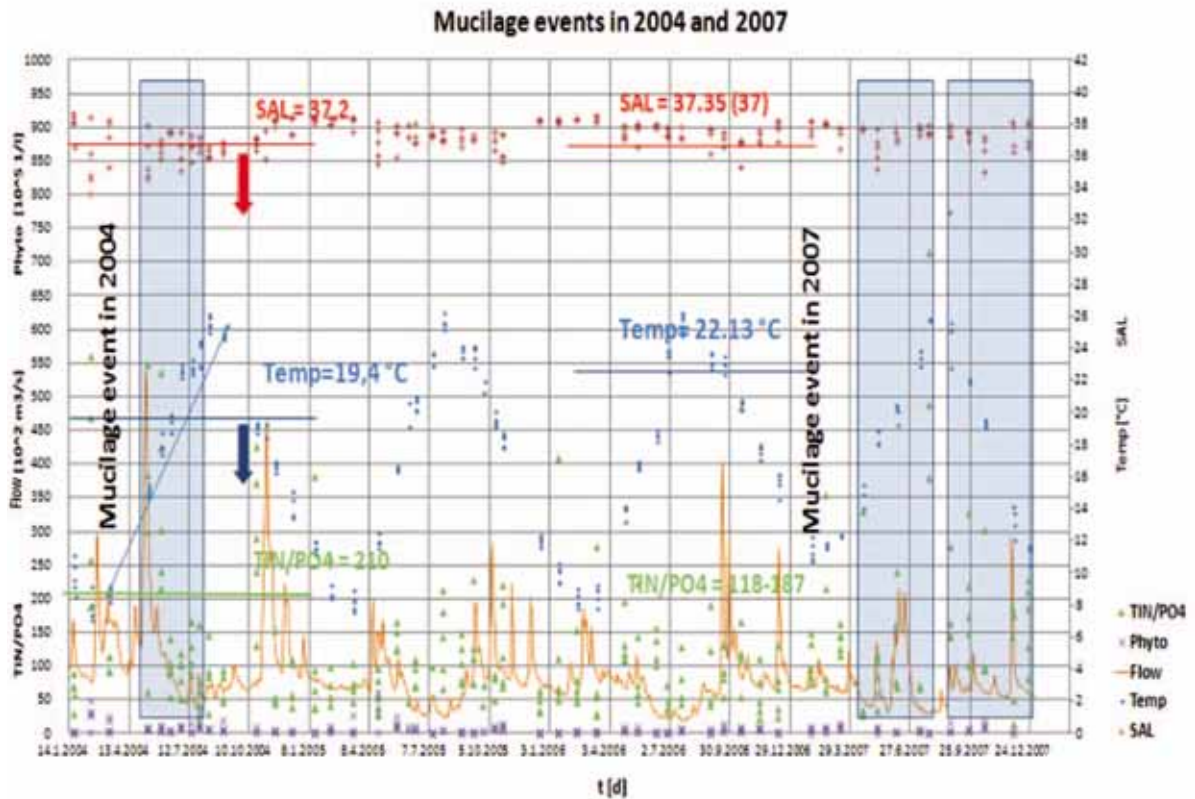


Fig. 9. Graphical presentation of TIN/PO₄ ratio, salinity, temperature, Po River flow and total phytoplankton with temporal occurrence of mucilage events in 2004 and 2007

CONCLUSIONS

All data indicate in general that mucilage occur at higher TIN/PO₄ ratio and confirm previous hypothesis made by MYKLESTAD (1995), GRILLI *et al.* (2005) and DEGOBBIS *et al.* (2005). The model also shows the complexity of the mucilage events. However it clearly indicates that substantial phytoplankton bloom should proceed to mucilage events with the formation of the organic matter. (TOTTI *et al.*, 2005).

With regard to the latest research presented in RICCI *et al.* (2014) which shows that DIN/TP ratio was the most significant factor in predicting mucilage appearance, whereas DIN/DIP (Dissolved inorganic nitrogen/Dissolved inorganic phosphorus) ratio showed frequent temporal variability and appearing as an inconsistent parameter future work will be focused to examining this finding with ML techniques.

ML technique was applied in this research on long-term measured data (1972-2007) in the NA to induce a descriptive TIN/PO₄ ratio model that could indicate the mechanisms of occurrence of the mucilage events.

The model for TIN/PO₄ ratio confirmed the assumption that the mucilage events are correlated with the values of this ratio in the system, i.e. they coincide with the high values of the TIN/PO₄ ratio. Four distinctive periods of mucilage appearance were identified: 1982-1999, 2000-2003, 2004 and 2005-2007. Additionally to the previous research, the model developed here gives an insight on the threshold values of salinity and water temperature, which trigger specific values of the TIN/PO₄ ratio, further related to mucilage events. It shows that P limitation can be the trigger of increased frequency of mucilage events.

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Studija utjecaja omjera TIN/PO₄ na pojavu cvjetanja mora u sjevernom Jadranu upotrebom regresijskih stabala

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SAŽETAK

Sjevero-zapadni dio sjevernog Jadrana (SJ) pokazuje eutrofne do mezotrofne karakteristike sa čestim pojavama cvjetanja algi te prilično nepredvidljivim cvjetanjima mora. Kako bi se doprinjelo razumijevanju cvjetanja mora u SJ upotrijebljen je algoritam strojnog učenja za izradu regresijskih stabala na skupu podataka koji sadrži fizičke, kemijske i biološke parametre mjerene na šest postaja na profile od delte rijeke Po (Italija) do Rovinja (Hrvatska). Upotrebom strojnog učenja izrađen je model koji opisuje vezu između omjera TIN/PO₄ koji se smatra neophodnim, a ponekad i glavnim okidačem za pojave cvjetanja mora te ekoloških uvjeta u SJ. Dobiveni model za omjer TIN/PO₄ potvrđuje pretpostavku da su pojave cvjetanja mora povezane sa danim omjerom, tj. pojave cvjetanja mora se podudaraju sa visokim vrijednostima tog omjera. Ovo otkriće ukazuje da se na određenim razinama ograničenja fosforom (vidljivo iz omjera TIN/PO₄) pojava frekvencije cvjetanja mora povećava. Model također otkriva da su salinitet i temperatura odgovorni za promjene omjera TIN/PO₄ te daje uvid u njihove granične vrijednosti koje dovode do visokih vrijednosti ovog omjera, a koji je dalje povezan sa pojavom cvjetanja mora.

Ključne riječi: omjer TIN/PO₄, cvjetanje mora, strojno učenje, regresijska stabla, sjeverni Jadran