

Use of artificial neural networks to identify the origin of green macroalgae

Radosław Żbikowski

Institute of Oceanography, University of Gdańsk, Av. Piłsudskiego 46, 81-378 Gdynia, Poland

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ABSTRACT

This study demonstrates application of artificial neural networks (ANNs) for identifying the origin of green macroalgae (*Enteromorpha* sp. and *Cladophora* sp.) according to their concentrations of Cd, Cu, Ni, Zn, Mn, Pb, Na, Ca, K and Mg. Earlier studies confirmed that algae can be used for biomonitoring surveys of metal contaminants in coastal areas of the Southern Baltic. The same data sets were classified with the use of different structures of radial basis function (RBF) and multilayer perceptron (MLP) networks. The selected networks were able to classify the samples according to their geographical origin, i.e. Southern Baltic, Gulf of Gdańsk and Vistula Lagoon. Additionally in the case of macroalgae from the Gulf of Gdańsk, the networks enabled the discrimination of samples according to areas of contrasting levels of pollution. Hence this study shows that artificial neural networks can be a valuable tool in biomonitoring studies.

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1. Introduction

Artificial neural network (ANN) research started in 1943 but a revival in their interest occurred since the late 1980's (Wu, 1994). ANNs represent a mathematical model emulating some properties of the human central nervous system and rely on adaptive learning and can be used to extract patterns and detects complex trends. There have been many papers that describe the theory of ANN in detail (e.g. Bishop, 1995; Livingstone, 2009). In general the networks are composed of a large number of highly interconnected processing artificial neurones working in parallel to solve a specific problem. The value of different networks can be related to their structure, dynamics and learning methods. The ANNs are also being successfully applied in environmental sciences (Kanevski et al., 1996; Reich et al., 1999; Dragovic et al., 2007; Musavi et al., 2007). In the shallow coastal zone of the Baltic Sea the green macroalgae *Enteromorpha* sp. and *Cladophora* sp. are often dominant benthic plants. They can be potentially used as valuable bio-indicators of environmental pollution because of their relatively wide distribution range in different coastal habitats, easy sampling and ability to absorb actively various contaminants from water, thus reflecting the ambient contaminant levels (Phillips, 1997; Sawidis et al., 2001; Villares et al., 2002). Earlier studies confirm that these species of green algae can also be used for biomonitoring surveys of metal contaminants in coastal areas of the Southern Baltic (Żbikowski et al., 2006, 2007). Moreover, green macroalgae have been used in various countries especially Asia as an important,

traditional food product (Mamatha et al., 2007). However, imported algae products may contain elevated levels of chemical contaminants and can be a high source of exposure for humans (Van Netten et al., 2000). Artificial neural networks have the ability to identify the origin of samples giving valuable information about green macroalgae quality and composition for consumption.

In the light of the aforementioned issues the obtained concentration of Cd, Cu, Ni, Zn, Mn, Pb, Na, Ca, K and Mg for *Enteromorpha* sp. and *Cladophora* sp. from the Southern Baltic, Gulf of Gdańsk and Vistula Lagoon was used to create neural networks. The main objectives of this work were to estimate of the value and compare the performance of RBF and MLP for: (1) determining the geographical origin of green macroalgae; and (2) determining anthropogenic pollution in the Gulf of Gdańsk.

2. Material and methods

2.1. Description of the study areas

The Baltic Sea is a non-tidal, semi-enclosed water body surrounded by highly industrialized countries. Three stations, i.e. Ustka, Jarosławiec, Darłowo are situated in coastal zone of the open Southern Baltic waters. This area is free from industrial inputs except sewage effluents. The Gulf of Gdańsk is a semi-closed basin of the southern Baltic. The concentration of pollutants in the gulf is strongly influenced by the inflow of the Vistula River, the largest river in Poland. Two major Baltic ports with the highest quantity of cargo transfer in Poland, i.e. Gdańsk and Gdynia, are located within the gulf and this brings a high risk of accidents and environmental

E-mail address: r.zbikows@wp.pl.

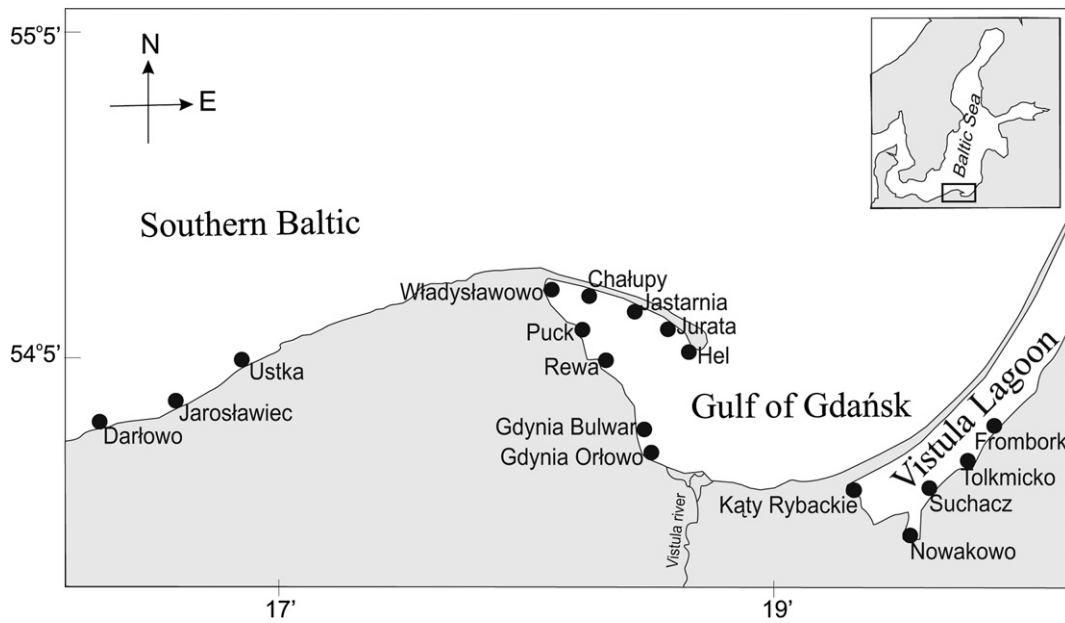


Fig. 1. Map showing location of the macroalgae sampling stations.

pollution from crude oil and other chemicals. Additionally the Gdańsk–Gdynia metropolitan area, with a total population exceeding 1 million, may be responsible for various interactions between anthropogenic activities in the region and the coastal ecosystem. Therefore, the Gdańsk region has been classified as one of the ecologically endangered areas in Poland and one of the pollution “hot spots” in the Baltic (HELCOM, 2003). The Vistula Lagoon with intermediate salinity i.e. 2–5 practical salinity units (Chubarenko and Tchepikova, 2001) is located in the south-eastern Baltic Sea. The lagoon is characterized by intensive eutrophication due to nutrient loads from urban areas, industry and agriculture. The importance of the lagoon is shown by its inclusion in the list of Baltic Sea Protected Areas (HELCOM, 1996).

2.2. Macroalgae material

The samples were hand-picked from the hard substrata at several stations in the coastal zone of the Southern Baltic (20 samples), Gulf of Gdańsk (128 samples) and Vistula Lagoon (13 samples) every month from May to October in 2000–2003 (Fig. 1). Prior to analysis the samples were washed with seawater in situ to remove trapped mud, sand, epiphytes and attached particles and then dried at 60 °C to a constant weight. All the samples were digested in analytically ultrapure HNO₃. Flame atomic absorption spectroscopy (FAAS) was used to measure the concentrations of chemical elements in the macroalgae. The results of multivariate factor analysis (FA) indicate the influence of natural environmental

or anthropogenic parameters on the chemical elements composition of *Enteromorpha* sp. and *Cladophora* sp. (Żbikowski et al., 2006, 2007). Due to the salinity effect, the macroalgae from the Southern Baltic contained more Na and K, while the anthropogenic impact of Cu, Pb and Zn was observed for the algae from the Gulf of Gdańsk in the vicinity of Gdynia metropolitan area. The earlier research revealed that even if one species was absent in the investigated area it is still possible to successfully conclude the biomonitoring studies by replacing the species by the second one, i.e. *Cladophora* sp. by *Enteromorpha* sp. and vice versa and in consequence still giving reliable results. Thus, in the present work the concentration values of chemical elements for each of the green algae were combined into the one data matrix.

2.3. Neural network models and software

In order to achieve the objective it was decided to employ two types of ANNs which are most commonly used in classification problems, i.e. multilayer perceptron (MLP) and radial basis function (RBF) networks (Bishop, 1995; Zhang, 2000; Ergun et al., 2004; Foody, 2004; Musavi et al., 2007). The general model of both networks consists of a number of nodes arranged in multiple layers, i.e. input, hidden and output layers with connections between the nodes in the adjacent layers by weights. In the case of MLP, the summation of each neuron j in the hidden layer by its input nodes x_i after multiplying the connection weights w_{ij} gives the output y_j as a function of the sum, that is:

Table 1

Concentration of heavy metals (mean ± SD, min–max, $N = 92$) and macroelements (mean ± SD, min–max, $N = 41$) in the green alga *Enteromorpha* sp. collected from the areas studied (Żbikowski et al., 2006).

	Heavy metals [$\mu\text{g/g d.w.}$]							Macroelements [mg/g d.w.]				
	N	Cd	Cu	Pb	Ni	Zn	Mn	N	Ca	Mg	Na	K
Southern Baltic	10	Not detected	3.78 ± 0.87 (2.34–5.50)	2.61 ± 0.71 (1.68–3.35)	3.84 ± 1.17 (1.82–5.53)	53.1 ± 16.5 (14.4–74.9)	151.0 ± 58.6 (65.8–213.3)	10	6.5 ± 2.8 (3.3–10.7)	14.7 ± 3.0 (10.3–20.2)	35.0 ± 10.5 (21.5–53.1)	52.7 ± 6.7 (42.3–63.0)
Gulf of Gdańsk	77	0.44 ± 0.24 (0.03–1.08)	4.92 ± 2.33 (1.82–11.60)	3.77 ± 2.17 (0.90–10.39)	3.61 ± 1.71 (0.13–7.09)	64.1 ± 40.5 (13.6–175.9)	172.7 ± 86.3 (30.7–384.1)	26	6.4 ± 3.1 (2.6–15.5)	18.0 ± 3.6 (12.3–25.1)	25.7 ± 7.7 (10.5–38.8)	35.8 ± 10.9 (19.7–62.3)
Vistula Lagoon	5	0.24 ± 0.18 (0.07–0.55)	4.61 ± 1.37 (2.79–5.48)	3.56 ± 0.83 (2.66–4.61)	8.20 ± 1.61 (5.80–10.20)	32.1 ± 12.0 (15.3–43.6)	361.5 ± 111.0 (220.1–499.9)	5	5.5 ± 1.0 (4.0–6.4)	19.5 ± 5.4 (12.5–25.7)	20.5 ± 6.5 (12.6–27.2)	26.4 ± 6.2 (17.0–32.8)

Table 2
Concentration of heavy metals (mean \pm SD, min–max, $N = 69$) and macroelements (mean \pm SD, min–max, $N = 39$) in the green alga *Cladophora* sp. collected from the areas studied (Żbikowski et al., 2007).

	Heavy metals [$\mu\text{g/g d.w.}$]							Macroelements [mg/g d.w.]				
	N	Cd	Cu	Pb	Ni	Zn	Mn	N	Ca	Mg	Na	K
Southern Baltic	10	Not detected	5.15 ± 1.28 (2.58–7.17)	4.35 ± 1.35 (1.41–6.20)	6.36 ± 2.21 (3.45–10.60)	67.5 ± 18.2 (41.6–94.1)	253.3 ± 104 (75.5–428)	10	4.5 ± 1.3 (2.2–6.8)	19.4 ± 5.7 (11.5–29.5)	37.3 ± 7.2 (23.4–47.5)	53.4 ± 11.7 (34.1–70.3)
Gulf of Gdańsk	51	0.29 ± 0.12 (0.08–0.62)	5.32 ± 1.99 (1.10–11.25)	5.11 ± 2.69 (1.81–13.20)	3.50 ± 1.87 (0.79–9.18)	63.0 ± 33.3 (21.7–146.4)	298 ± 161 (48.7–740)	21	3.9 ± 2.2 (1.2–9.2)	15.0 ± 6.1 (4.4–24.0)	20.9 ± 8.9 (3.9–39.1)	38.5 ± 8.0 (24.3 \pm 55.3)
Vistula Lagoon	8	0.19 ± 0.09 (0.11–0.39)	8.36 ± 3.09 (3.81–11.42)	7.46 ± 3.76 (2.66–14.55)	11.42 ± 3.99 (5.75–17.01)	73.1 ± 27.6 (30.0–105)	1185 ± 450 (712–1930)	8	5.4 ± 2.6 (2.5–10.0)	14.9 ± 7.4 (4.1–28.8)	17.1 ± 7.2 (6.1–27.4)	30.7 ± 8.7 (21.2–48.4)

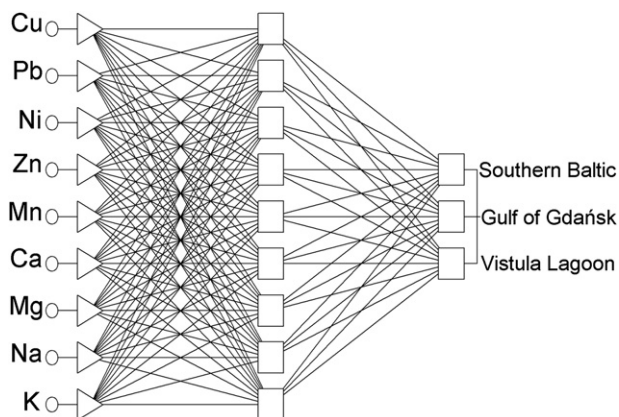


Fig. 2. Architecture of best MLP network with sigmoidal activation function used in classification analysis according to the macroalgae geographical origin.

Southern Baltic, Gulf of Gdańsk and Vistula Lagoon. The contents of heavy metals in algae samples from the Gulf of Gdańsk were used to assess anthropogenic pollution of the area. In this case, input parameters affected the activation of one output neuron-producing signal indicating that the sample belongs to more (Gdynia stations) or less polluted areas (other stations), respectively.

The nets were created using data mining software package in Statistica for Windows (Release 7.1, Copyright© Statsoft, Inc.) with an effective tool as the intelligent problem solver, which can automatically evaluate a large number of different neural networks and select the most efficient one for the problem.

2.4. Data processing

The concentration data for the macroalgae were randomly divided into training, validation and test sets amounting to 50, 25 and 25%, respectively. For the geographical classification of algae the above subsets were composed of 81, 40 and 40 cases (samples) whereas for pollution model (concentration data from the Gulf of Gdańsk) of 64, 32 and 32 cases. In order to obtain a better classification performance of nets and to prevent them from a priori giving more importance to some elements based on their concentrations, we applied a min–max scaling before feeding into ANNs (Kanellopoulos and Wilkinson, 1997). The outputs corresponding to class membership of single data were coded in binary format 1-0. In the training phase, the correct class for each sample set was known, so-called supervised training, and the output nodes can therefore be assigned. The validation set was used to assess the quality of the net during the training process and the test set was used for evaluation of the established model after the training phase. MLP networks were trained using a back-propagation algorithm (Leonard and Kramer, 1990). The idea of the back-propagation method is to propagate the error back through the network and to adjust the weights of each layer as it propagates. One complete presentation of training set is called an epoch. On each epoch, the data were submitted in turn to the network, the error was calculated and the weights were adjusted and then the process repeats. In this work 100 epochs were set by hand and the program reaches a satisfactory solution. A known problem in training MLP is that the training

$$y_j = f\left(\sum w_{ji}x_i\right) \quad (1)$$

where f is the sigmoidal transfer function. The parameters of an RBF type neural network consist of the centers U_j and the spread σ_j of the basis functions at the hidden layer nodes and the synaptic weights w_{kj} of the output layer nodes. For an input vector X^i , the j hidden node produces a response h_j given by:

$$h_j = \exp\left\{-\frac{\|X^i - U_j\|}{2\sigma_j^2}\right\} \quad (2)$$

where $\|X^i - U_j\|$ is the distance between the point representing the input X^i and the center of the j hidden node as measured by some norm.

In this study, input nodes are attributable to values for each of the chemical elements, hidden layers are used for computations and output layers correspond to the recognized sampling areas. The data for the macroalgal concentrations of macroelements and heavy metals were used to determine their geographical origin, i.e.

Table 3
The classification results of *Enteromorpha* sp. and *Cladophora* sp. samples from the Southern Baltic ($N = 20$) Gulf of Gdańsk ($N = 128$) and Vistula Lagoon ($N = 13$) using MLP and RBF networks on the basis of the chemical elements content.

Network	Area	Training set		Validation set		Test set	
		Correctly classified (%)	Misclassified (%)	Correctly classified (%)	Misclassified (%)	Correctly classified (%)	Misclassified (%)
MLP	Southern Baltic	77	23	100	0	100	0
	Gulf of Gdańsk	96	4	100	0	87	13
	Vistula Lagoon	83	17	75	25	70	30
RBF	Southern Baltic	90	10	100	0	100	0
	Gulf of Gdańsk	91	9	77	23	74	26
	Vistula Lagoon	100	0	68	32	100	0

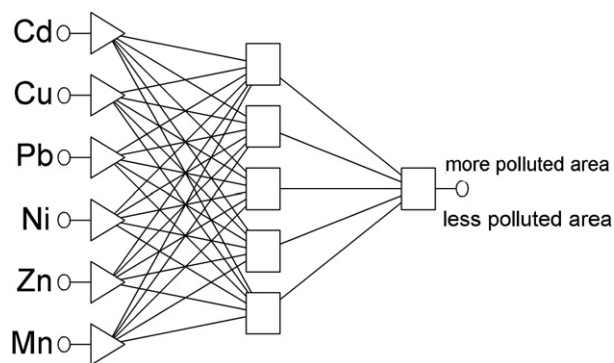


Fig. 3. Architecture of best MLP network with sigmoidal activation function used in classification analysis of macroalgae according to more or less polluted areas of the Gulf of Gdańsk.

process is trapped in a local minimum instead of reaching a global minimum. A parameter called the momentum term which causes the weight changes to be affected by the size of the previous weight changes is used to avoid the local minima (Bishop, 1995; Nisbet et al., 2009). The learning rate tells the network how slowly to progress. In this work a learning rate and momentum were kept constant during training and were set to 0.1 and 0.3, respectively. RBF networks training process were conducted with the use of K-means and K-nearest neighbor algorithms and the output layers were optimized using the pseudo-inverse method (Wu, 1994).

2.5. Network parameters

Approximately 3000 artificial neural network configurations were tested and the best were selected. The structures of artificial neural networks selection criteria were based on the percentage of correctly classified samples and values of the root mean square (RMS) error of validation and test data sets. The model characterized by the highest percentage and the smaller RMS error was selected.

The quality of nets with one output node was also estimated on the basis of value for the area under the receiver operating characteristic (ROC) curve. ROC curves quantify the overall ability of the designed model to discriminate between two classes. An area under the ROC curve of 0.5 is equivalent to chance discrimination and an area amounting to 1.0 suggests a perfect model because it achieves maximum sensitivity (true positive rate) and specificity (true negative rate) (Meistrell and Spackman, 1989).

The other important neural network parameter, i.e. sensitivity analysis, was also carried out to assess the relative importance and relationship between the inputs and outputs of the neural network. In models involving many input variables the investigation is a relatively effective method and an essential component for model building and quality assurance. Based on the sensitivity values, the ranking of all input parameters for each chemical element was established. A value greater than 1 indicates the higher ranking of

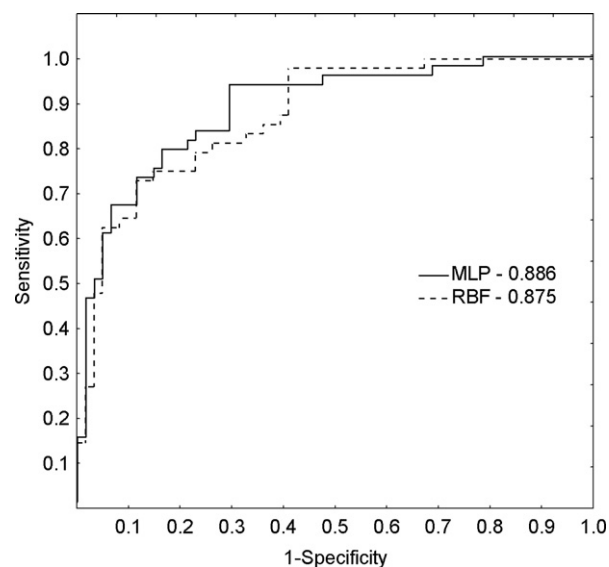


Fig. 4. ROC curve for MLP and RBF networks.

input parameter while less important input variables are represented by the value less than 1 (Yeung et al., 2009).

3. Results and discussion

The concentrations of chemical elements in macroalgae (Tables 1 and 2) were presented to the MLP and RBF networks, which categorize them according to three classes of the sampling sites, i.e. Southern Baltic, Gulf of Gdańsk and Vistula Lagoon. The most suitable structure of MLP network is presented in Fig. 2. The topology of best RBF was similar to MLP but consisted of 8 rather than 9 nodes in the hidden layer. In the case of both networks the calculated validation and test (in parentheses) RMS errors were similar and as follows: MLP – 0.207 (0.268); RBF – 0.225 (0.214). Table 3 shows that a comparable satisfactory classification accuracy was achieved by the neural networks. In the case of MLP net the number of correctly classified samples varied between 75 and 100% for the verification and 70 and 100% for the test data.

An earlier study indicated the influence of anthropogenic parameters on the chemical elements composition of *Enteromorpha* sp. and *Cladophora* sp. samples from the Gulf of Gdańsk (Żbikowski et al., 2006, 2007). The heavy metal concentration of macroalgae samples was presented to the MLP and RBF networks, which categorize them according to more (Gdynia samples) or less (other gulf samples) polluted areas of the Gulf of Gdańsk. The results revealed that a trained network can be applied to data obtained from biomonitoring studies and it can provide a suitable information on the present status of heavy metal pollution in individual area of the gulf. Fig. 3 shows the architecture of the best MLP trained with the back-propagation algorithm (47 epochs). The RBF

Table 4

The classification results of *Enteromorpha* sp. and *Cladophora* sp. samples from the less polluted ($N = 71$) and more polluted ($N = 57$) areas of the Gulf of Gdańsk using MLP and RBF networks on the basis of the chemical elements content.

Network	Area of gulf	Training set		Validation set		Test set	
		Correctly classified (%)	Misclassified (%)	Correctly classified (%)	Misclassified (%)	Correctly classified (%)	Misclassified (%)
MLP	Less polluted	83	17	93	7	70	30
	More polluted	80	20	86	24	90	10
RBF	Less polluted	92	8	74	26	69	31
	More polluted	77	23	70	30	75	25

Table 5
The networks sensitivity values and their ranking (in parentheses) of input variables contribution for the classification.

	Cd	Cu	Pb	Ni	Zn	Mn	Ca	Mg	Na	K
Southern Baltic, Gulf of Gdańsk, Vistula Lagoon										
MLP		1.030 (6)	1.008 (8)	1.129 (4)	1.248 (2)	1.091 (5)	1.023 (7)	0.938 (9)	1.200 (3)	1.286 (1)
RBF		1.002 (7)	1.001 (8)	1.105 (2)	1.014 (5)	1.046 (4)	1.004 (6)	0.989 (9)	1.087 (3)	1.152 (1)
Gulf of Gdańsk										
MLP	1.004 (2)	1.107 (1)	1.000 (4)	1.001 (3)	0.959 (6)	0.973 (5)				
RBF	0.973 (6)	1.064 (1)	0.975 (5)	1.032 (4)	1.043 (3)	1.049 (2)				

model consisted of the same number of neurons in each layer. The estimated validation and test RMS errors for the MLP (0.237 and 0.245 respectively) were comparable to those obtained for RBF (0.249 and 0.254 respectively). As shown in Table 4, both networks tend to be an efficient tool for classifying of green macroalgae origin. In the case reported here, the MLP model seems to outperform RBF in terms of percentage of correctly classified samples on the validation set although the difference is not large. The quality of ANNs with one output node was assessed on the basis of value for the area under ROC curve (Fig. 4). The closer to the left-hand border and then the top border of the ROC space the curve follows, the more accurate the model (Hanley and McNeil, 1982). The results show that comparable and satisfactory values were achieved by the two neural networks in the case of the ROC parameters, i.e. 0.886 for MLP and 0.875 for RBF. The graph shows the trade-off between sensitivity and specificity, i.e. any increase in sensitivity will be accompanied by a decrease in specificity. It is important to note that sensitivity (true positive rate) and specificity (true negative rate) only provide information on the proportion or percentage of samples from more or less polluted areas which are correctly categorized. Fig. 4 shows that sensitivities at 80% and 90% specificity for MLP (80 and 68%) were acceptable and similar than those for RBF (75 and 62%).

ANN sensitivity analysis was carried out to estimate the importance of individual chemical elements in the classification networks (Table 5). It is of note that most of the chemical elements (values above 1) tend to be important input variables. The data obtained suggest that in the case of both multiple output networks K, due to salinity differences between areas, was the most important variable in the classification of green macroalgae. The highest concentrations of K were noted in macroalgae from the Southern Baltic while the lower and lowest ones in the algae from the Gulf of Gdańsk and Vistula Lagoon, respectively. For one output model, Cu, which is possibly to be anthropogenic in origin, is a significant element which controls the classification of samples from the Gulf of Gdańsk (Żbikowski et al., 2006, 2007). Balbinot et al. (2005) also noted that a designed neural network shows that concentration of Cu in macroalgae can be used for screening purposes.

There are several well known and widely used chemometric methods that provide spatial estimates and/or predictions according to chemical element data obtained from environmental monitoring studies (Szefer, 2003). The advantage of ANN is the ability to learn many new relationships without necessarily forgetting things learned in the past. If in the future a new large data set becomes available we can feed the designed neural model with new data and retrain the network. In this case the network instantly improves its predictive ability. Thus, as an example, the assessment of complex anthropogenic impacts can be improved substantially and managed more efficiently using the neural-based approach. Moreover, it is of note that the application of ANN in extensive biomonitoring studies can reduce the costs connected with the necessity of carrying out further time-consuming environmental surveys.

There are different ways to build appropriate MLP and RBF models for the classification of problems (Bishop, 1995; Zhang,

2000). Although many studies containing quite sophisticated solutions have been proposed, the comparison of the approaches presented in the literature is sometimes difficult. This is because conditions differ in each case and most of the articles present a specified solution to the problem, therefore direct comparison of the obtained errors and percentage of classified samples tends to be quite meaningless. The most important decisions concern the selection of the input variables and network architecture. In general, RBF networks have the advantage that they can be developed and implemented with much less time and effort when compared to the MLP networks (Jayawardena et al., 1998). The limitation of the RBF neural network is that it is more sensitive to dimensionality and has greater difficulties if the number of units is large. A further disadvantage is that the RBF network should be adapted to an individual application, whereas the MLP network is suitable for a number of different applications because its learning strategy is more complex (Livingstone, 2009; Nisbet et al., 2009). The results here indicate that both designed and implemented MLP and RBF models are robust classifiers with the ability to assess geographic macroalgal origin and quality of the habitat area.

There are some countries where green macroalgae, especially *Enteromorpha* sp., are an important consumed food product, which is exported worldwide (McHugh, 2003). The assessment of geographic origin and identification of product authenticity with the use of neural networks have been conducted successfully by various authors in food science (Herrador and Gonzalez, 2001; Alcazar et al., 2002; Hernandez-Caraballo et al., 2003; Perez-Magarino et al., 2004; Moreno et al., 2007). Thus, the application of artificial neural networks and the acquired knowledge about the macroalgal origin can be important in controlling their appropriate quality and composition for consumption.

There are only a few studies that have employed the chemometric approach to describe the correlation between locations and chemical element contamination of biological material in which ANNs were used (Kanevski et al., 1996; Balbinot et al., 2005; Samecka-Cymerman et al., 2007). Dragovic et al. (2007) found that an adequate classification of moss and lichen samples of different origin could be achieved by applying neural networks on radionuclide data obtained from biomonitoring studies. Balbinot et al. (2005) observed that the use of the designed neural network facilitates data interpretation from environmental samples, quality-control processes and diagnoses.

4. Conclusions

The results revealed that the obtained MLP and RBF neural networks are effective tools for identifying the geographical origin of green macroalgae. The chemical element contents of *Enteromorpha* sp. and *Cladophora* sp. are suitable descriptors for differentiating amongst the Southern Baltic, Gulf of Gdańsk and Vistula Lagoon. Additionally, the application of the neural networks to biomonitoring data allows the identification of green macroalgae samples affected by contaminants and it may be useful in assessment of the quality of the study area.

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