**Macroinvertebrates as indicators of xenobiotics in river basin - ANN based approach**

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**Abstract**

Estimating the ecological condition of long rivers, such as the Danube River, remains a significant challenge. Xenobiotics, including pesticides, which are hazardous substances originating from human activities, can pose a serious threat to aquatic ecosystems. Developing a methodology that can promptly provide an information about the ecological state of a river, in line with the amount of such particular xenobiotics, represents a significant advantage in the field of environmental engineering.

This paper presents an advanced model of an Artificial Neural Network (ANN) that efficiently identifies the occurrence of particular xenobiotics in river monitoring procedures, while closely aligning with the structure of macroinvertebrate communities as reliable biomarkers. Biomarkers, as early indicators of the presence and/or effects of chemical stress, can significantly contribute to the identification of priority sites, enabling the establishment of clear causal relationships between hazardous substance pollution and changes in aquatic communities. The application of bioanalytical methods, predominantly *in vitro*, and the deployment of biomarkers in showing risk assessment offer a capable solution to overcome this challenge. Macroinvertebrates, proposed as official biological parameters for predicting the ecological state of rivers according to the Water Framework Directive (WFD), exhibit diverse responses to chemical stressors. By establishing the appropriate relationship linking specific biological and chemical parameters, it is possible to develop an Artificial Intelligence (AI)-based model that can forecast the ecological state of a river *in situ* with a high degree of precision measured by Mean Squared Error (MSE). This correlation-driven AI model can provide reliable and appropriate assessments of the river's ecological condition, allowing for effective monitoring and management of aquatic ecosystems. In line with some previous studiesparticular xenobiotics, mostly classified as pesticides: 2,4-Dinitrophenol, Chloroxuron, Bromacil, Fluoranthene and Bentazon exhibit the most pronounced correlate

ion with macroinvertebrate communities within the Danube River basin.

The establishment of a robust relationship between specific xenobiotics and macroinvertebrates served as an initial step in developing an Artificial Neural Networks (ANN) model suitable for future research in the field of ecological engineering. Established Artificial Neural Network (ANN) based models, one for each group of xenobiotics, demonstrated exceptional accuracy and emerged as effective, cost-efficient, and sustainable tools for predicting the ecological state of rivers and other surface water bodies with determined selected species of macroinvertebrates. These models are particularly valuable for predicting the ecological state in relation to specific selected species of macroinvertebrates, providing valuable insights into the health and dynamics of these aquatic ecosystems. The performance of the ANN models was assessed through the Mean Squared Error (MSE).

1. **Introduction**

Aquatic ecosystems are continuously exposed to multistress conditions corresponding to simultaneous impacts from various sources, including global changes, hydromorphological modifications of water bodies and pollution originating from concentrated and diffuse sources. In multistress conditions, achieving the effectiveness of management measures to maintain or enhance the status of aquatic ecosystems heavily depend on the precise assessment of risks and the identification of significant pressures. Due to the complex nature of multistress conditions this task involves significant challenges. Predicting the ecological state of the Danube River, recognizable as both an international waterway and the longest river within the European Union, continually presents a challenging task. In line with the Water Frame Directive (WFD), the most significant section of European water legislation in last two decades, both macroinvertebrates and concentrations of chemical compounds, such as xenobiotics, are official parameters for ecological state prediction of surface waters **[1]**. Xenobiotics contain a wide range of substances, including environmental pollutants like industrial and agricultural chemicals, heavy metals, pharmaceuticals and compounds with hormonal activity. In monitoring of aquatic ecosystems these substances are known as chemical stressors. The presence of xenobiotics in surface water assess direct impact of those organic contaminants on water quality and ecosystem health **[2,3]**. Exposure to subtoxic concentrations of those chemicals as an immediate consequence follow a damage of the DNA molecule. The alterations in DNA induced by xenobiotics hold significant implications as they endanger not just individual cells and organisms, but entire populations by disrupting their genetic makeup. Consequently, the assessment of pollutant-induced DNA damage plays a vital role in environmental risk evaluation **[4]**. Macroinvertebrate communities in river courses may perform as bioindicators sensitive to environmental changes with various values of ecological valence to chemical exposures. Environmental variables, such as the presence of various chemicals in water, can significantly influence the composition and structure of macroinvertebrate fauna**[5]**.Both physical and chemical methods offer effective information about water quality at specific moments, while biological monitoring provides a holistic and integrated identification of water conditions over time. The dynamic nature of running waters provides macroinvertebrates with the exceptional ability to capture and reflect the health and long-term trends of aquatic ecosystems, making them a valuable tool for ecosystem assessment **[6]**. At the outset of this research, establishing a correlation among the presence of specific macroinvertebrate species and the concentrations of chosen xenobiotics serves as the initial focal point. Developing a model of Artificial Neural Networks (ANN) based on those facts of macroinvertebrates / xenobiotic dependency in future scientific investigations may perform as an appropriate tool in fields of ecological engineering and river management. Improved model of ANN with distinctly defined relations among inputs and outputs in future surveys of river ecological state will be capable to provide an information of specific xenobiotic concentration in accordance to presence of macroinvertebrate species in the sampling site. The utilization of an ANN-based model offers several crucial advantages, including its cost-effectiveness and eco-friendly nature, making it a suitable method for *in situ* prediction of river sampling sites. Employing this type of river monitoring approach certainly promotes sustainability by providing a high level of accuracy in predicting the ecological state. ANN based modeling of river ecological state provide developing a model that certainly can be used in future investigations and river ecological state prediction, limited only to robustness of dataset. The quantity of data incorporated into modeling process significantly influences the level of accuracy in prediction. A well-developed model is the most suitable for accurately modeling the ecological status of rivers that are as long as, or at least comparable in length to, the Danube River.

Building upon the developed ANN-based model, there is a potential to create a web tool capable of forecasting the concentration of xenobiotics by analyzing the presence of macroinvertebrates as indicators.

1. **Study area and field survey data**

In aim to undertake an international longitudinal survey that will provide reliable information of water quality for the whole length of the Danube River, including major tributaries, starting from 2001 on every six years were organized Joint Danube Survey (JDS) expeditions. During JDS scientific expeditions, comprehensive datasets were gathered, containing all the official biological, physico-chemical, and hydromorphological parameters specified by the Water Framework Directive. These datasets form the foundation for the proposed ecological state prediction framework **[1,7]**.

To support this research two sets of official biological and chemical parameters were extracted from the dataset collected during the Joint Danube Survey 3 (JDS 3) scientific expedition for the purpose of predicting the ecological state of rivers: the presence of particular macroinvertebrates and the concentrations of specific xenobiotics **[8]**. A total of 68 sampling sites were selected along a 2581 km stretch of the Danube River, covering both left and right banks of the river **(Figure 1.)** The survey encompassed 3 kilometers on each side of the river, amounting to a total of 6 kilometers sampled at each sampling site in the main channel. Biological parameters were sampled together with the water samples collected directly from the river **[9]**.



**Figure 1.** Map and scheme of the macroinvertebrate and xenobiotics sampling sites

1. **Materials and methods**
   1. **Dataset**

With the goal of creating an Artificial Intelligence-based model with exceptional predictive accuracy, samples lacking recorded macroinvertebrate species were excluded from the analysis.

Species with broad tolerances to environmental changes and high saprobic valencies were similarly omitted from the study. In order to preselect the input and output variables for the model a Biological Environmental (BIO-ENV) gradients analysis results were used to examine the correlation between the macroinvertebrate community and chemical pollutants **[8]**. The most influential from 20 prioritized River Basin Specific (RBS) chemical pollutants for the Danube River corresponding to **Slobodnik et al [9]** in the water were identified using Forward Selection (FS) method based on Pearson’s correlation test (p<0.05) and the Monte Carlo permutation test. The Forward Selection (FS) results revealed significant correlations between macroinvertebrate communities along the Danube River basin and several chemical pollutants, including Chloroxuron, Bentazon, Bromacil, Dimefuron, 2.4-Dinitrophenol, Amoxicillin, and Fluoranthene **[10]**.

Canonical Correspondence Analysis (CCA) was employed to determine the key input variables for the Artificial Neural Network (ANN) models. Based on CCA results, particular, numerically determined, macroinvertebrate species presence were selected as input variables for developed ANN models. Specifically, those are species that show tolerance to Bromacil, 2.4-Dinitrophenol, Bentazon, Fluoranthene, as well as those that are tolerant to both Fluoranthene and 2.4-Dinitrophenol **(Table 1.)** The chosen outputs contain the concentrations of specific xenobiotics that demonstrate statistically established correlations with the input variables.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Subclass of selected species | Species tolerant to Bromacil | Species tolerant to 2.4-Dinitrophenol | Species tolerant to Fluoranthene | Species tolerant to both Fluoranthene and 2.4-Dinitrophenol | Species tolerant to Bentazone |
| Bivalvia | *Pisidium supinuim;*  *Pisidium henslowantum;*  *Pisidium nitidum;*  *Pisidium casertanum;*  *Pisidium moitessierianum;*  *Pisidium subtruncatum* | *Corbicula fluminea* | *Dreissena sp.;*  *Dreissena bugensis Andrusov, 1897; Sinanodonta woodiana;*  *Pisidium amnicum Müller, 1774****;***  *Sphaerium corneum* | *Unio pictorum;*  *Unio tumidus;*  *Corbicula fluminalis; Sphaerium rivicola Lamarck, 1818* | *Corbicula fluminea;* |
| Gastropoda | *Theodoxus fluviatillis);*  *Viviparus viviparus* | *Lythoglyphus naticoides;*  *Physella acuta* | *Theodoxus danubialis Pfeiffer, 1828;*  *Viviparus acerosus Bourguignat, 1862* |  |  |
| Oligochaeta | *Nais barbata;*  *Nais elunguis* | *Nais communis Piguet, 1906* | *Tubifex tubifex;*  *Limnodrilus udekemianus* | *Limnodrilus hoffmeisteri Claparede, 1862;*  *Limnodrilus claparedeanus Ratzel, 1868; Branchiura sowerbyi* |  |
| Crustacea |  | *Chelicorophium curvispinum L;*  *Dikerogammarus haemobaphes Eichwald, 1841* | *Dikerogammarus sp.;*  *Dikerogammarus bispinosus;*  *Dikerogammarus villosus;*  *Chelicorophium sp.* |  |  |
| Chironomidae |  | *Polypedilum scalaneum* |  |  | *Tanytarsus sp* |
| Odonata |  | *Gamphus flavipes;*  *Gamphus vulgatissimus L.* |  |  |  |

**Table 1.** Selected macroinvertebrate species tolerante to specific xenobiotics

List of the selected RBS pollutants for the Danube River according to **[9]** with the minimum and maximum recorded concentrations and the standard deviations (St.Dev.) are presented in **[10]**

|  |  |  |  |
| --- | --- | --- | --- |
| Substance (µg/l) | Min | Max | St.Dev. |
| 2,4-Dinitrophenol (DNP) | 0.01 | 0.04 | 0.007583024 |
| Bromacil | 0.0231236 | 0.186626 | 0.03574977 |
| Fluoranthene | 0.002 | 0.0204 | 0.002809182 |
| Bentazon | 0.003 | 0.02 | 0.002485741 |

* 1. **Data analysis**

Inspired by biological neural network, Artificial Neural Networks (ANN) represent a robust computational technique used for modeling complex non-linear relationships.

In order to develop an accurate model of Artificial Neural Networks for predicting the ecological state of a river basin, based on the presence of macroinvertebrates and chemical parameters, we completed the selection of input and output variables by considering the results of statistical analyses obtainable by **Popović et al. [10]**.

Through the implementation of the Forward selection (FS) method and Monte Carlo test assessment, seven out of the twenty analyzed River Basin Specific (RBS) pollutants were identified as the most influential chemical variables. These variables were found to be significantly correlated with the macroinvertebrate communities along the Danube River.

To facilitate the development of an Artificial Neural Network (ANN) model capable of accurately predicting the ecological state of a river basin using macroinvertebrate communities and concentrations of selected RBS pollutants, a total of five models were created, each of them having identical architectures. In the conducted investigation, numerical data on the presence of selected macroinvertebrates at all JDS sites were employed as input variables for the ANN modeling. The output variables, consisting of concentrations of xenobiotics Bromacil, 2.4-dinitrophenol, Fluoranthene and Bentazone, were selected based on FS statistical analysis, which indicated specific macroinvertebrate species' tolerance to these compounds **[11,12]**. Furthermore, a particular ANN model was employed, incorporating concentrations of both Fluoranthene and 2.4 - dinitrophenol as the output variables. Following this approach, a diverse assemblage of xenobiotics-tolerant macroinvertebrate species was employed as the input data. Including various neural network types, the Multi-Layer Feed-Forward Neural Network (MLFFNN) is the most popular and commonly used model for diverse tasks **[13]**. The MLFFNN architecture comprises a layered feedforward neural network, with neurons arranged in successive layers and information flowing unidirectionally from the input layer to the output layer through the hidden layer(s). The signal passing through the neuron is altered by weights and transfer functions. The number of input and output units depends on the input and output object representations, respectively. The neural network model is trained using a set of observed input and output values (training data set) and subsequently validated with another, to neural network unknown, data set (test set). In this study, five multilayer feed-forward neural networks with backpropagation learning were created to predict the river water quality in accordance with specific xenobiotic concentration levels.

 The ANN model consisted of four layers: an input layer including 106 neurons (macroinvertebrate species); two hidden layers (12 and 30 neurons) and one output layer consisted of 1 or 2 neurons, depending on number of included xenobiotics in the output layer. In an Artificial Neural Network, the output of a particular layer is obtained by calculating the sum of the products of inputs and their corresponding weights, followed by the application of an activation function, mathematical equation used for the purpose of transforming an input to output signals. The activation function utilized for the hidden layers was the sigmoid function, while the output layer employed the linear function for activation. The training of the ANN model utilized the Adam optimizer with a learning rate of 0.001. During the training process, were completed a total of 200 epochs, with a batch size 10.

A total of 5 neural networks with the same architecture were created, a one for each specified combination of inputs and outputs.

For model accuracy evaluation was used a common approach established on Mean Squared Error value.

Modelling was carried out using the Python programming language and Keras library **[13,14]**.

1. **Results** 
   1. **Performance evaluation**

In the realm of model evaluation, there is a wide range of statistical criteria available to assess the adequacy and effectiveness of a given model. In the present study, the performance evaluation statistics employed for training artificial neural networks (ANNs) include Mean Squared Error (MSE) determined by following equation: MSE = (1/n) · Σ (yi - ŷi)², where n is the number of observations or data points, yi is the actual or observed value and ŷi is the predicted value **[14]**.

In the domain of evaluating ANN performance, a lower Mean Squared Error (MSE) indicates a superior model fit. A reduced MSE implies that the model's predictions align closely with the actual observed values, demonstrating improved accuracy and performance of the ANN.

* 1. **The model prediction performances and validation**

An individual Artificial Neural Network (ANN) model was created and applied to each of the selected output parameters. In total, five different models, with particular xenobiotic concentration as an output (Bromacil, 2.4-Dinitrophenol, Fluoranthene, Bentazon and both 2.4-Dinitrophenol and Fluoranthene), were generated, trained and tested. The best prediction performances were achieved for the model based on particular species of subclasses *Bivalvia* and *Oligochaeta* **(Table 1.)** recognized as tolerant to both 2.4-Dinitrophenol and Fluoranthene as inputs. Estimated Mean Squared Error (MSE) for this model is 1.45 ·10-5. Results for each of developed ANN models based on values of MSE are presented in **Table 3.**

|  |  |
| --- | --- |
| **ANN model outputs** | **Mean Squared Error** |
| Bromacil | 0.0125 |
| 2.4-Dinitrophenol | 0.0001 |
| Fluoranthene | 0.0004 |
| 2.4-Dinitrophenol and Fluoranthene | 1.45 ·10-5 |
| Bentazone | 0.0012 |

**Table 3.** The Mean Squared Error (MSE) values obtained from the developed Artificial Neural Network (ANN) models

The presence of pollutants directly affects the primary production in aquatic ecosystems and reduces the possibility of survival of organisms. There is a disturbance of the balance of complex biotic relationships of macroinvertebrates. Based on the obtained results, a variety of taxa, such as species belonging to Oligochaeta (Annelida), Chironomidae (Insecta: Diptera) and Mollusca, were present in occurrences of different pollutants. The presence of organic compounds in aquatic ecosystems could cause changes in community structure. Pesticides have many possible sources and are generally considered as pollutants that possess the potential to decrease the relative abundance as well as number of sensitive taxa in macroinvertebrate communities **[16]**.

1. **Discussion**

The macroinvertebrate species that are commonly recognized as indicators of the presence of the herbicide Bromacil, widely used in agricultural practices, mainly belong to the *Pisidium* species within the subclass Bivalvia. The exceptional accuracy attained in the ANN modeling (MSE = 0.0125) provides compelling evidence for the efficiency of these species as dependable bioindicators for identifying the presence of the respective herbicide at detected levels ranging from 0.023 to 0.186 µg/L. A particular pollutant can have a wide range of effects, varying from the decline of populations to the displacement of species. Further confirmation of the role of selected species of class Bivalvia as effective bioidicators of herbicide pollution lies in the observation that none of those species were found on sampling sites with lack of xenobiotic Bromacil. The strong correlation observed between the presence of those Bivalvia species and the existence of Bromacil underlines their exceptional reliability as indicators of herbicide contamination in aquatic environments. The application of the FS method specifically singled out *Corbicula fluminea* from the subclass Bivalvia and *Tanytarsus sp*. from the Chironomidae subclass, as the identified species displaying tolerance to Bentazon. The established Artificial Neural Network (ANN) model, utilizing the presence of those two remarkable species as inputs, and the concentrations of Bentazon as outputs, exhibited exceptional accuracy. The model achieved notably low Mean Squared Error (MSE) values, specifically recording MSE value of 0.0012, further emphasizing its reliability and precision in predicting Bentazon concentrations. Lengthwise the whole Danube River basin the concentration of Bentazon exhibits in a range of 0-0.008 µg /l, with a prevailing concentration level of 0.004 µg l. These remarkable macroinvertebrate species demonstrate a significant resilience to the effects of Bentazon, making them reliable bioindicators for the presence of this herbicide in aforementioned concentrations in aquatic ecosystems. Even though that FS statistics didn’t recognize species from subclass *Oligochaeta* (class *Clitellata*, phylum *Annelida*) as tolerant to Bentazon, after including those particular species in addition to other inputs no differences in MSE scores were observed. Significant species of *Oligochaeta* obtained in this study are recognized for their tolerance to low concentrations of dissolved oxygen and increased pollution. Therefore, the presence of species from this subclass can serve as reliable indicators of the trophic stages of a river and indicate potential interventions to achieve improved ecological outcomes.

2.4-dinitrophenol (2.4-DNP) is a phenolic compound that finds applications as both a wood preservative and a pesticide, and also presents significant hazards to freshwater organisms. Despite the ecological risks associated with 2.4-DNP, only a limited number of studies have investigated its effects on aquatic environments**.** Despite the well-established acknowledgement of 2.4-Dinitrophenol (2,4-DNP) as highly toxic to human health at concentrations exceeding 400 μg/L, guideline values aimed at protecting aquatic organisms from the adverse effects of this compound are very limited. This discrepancy highlights the need for further research and the establishment of comprehensive guidelines to safeguard the well-being of aquatic ecosystems and the organisms inhabiting them **[17]**.

Tolerance to 2.4-Dinitrophenol has been observed in species belonging to six different subclasses, namely Bivalvia, Gastropoda, Oligochaeta, Crustacea, Chironomidae and Odonata. The observed Mean Squared Error (MSE) of 0.0001 for the ANN model, based on the mentioned input/output combination, signifies the model's exceptional accuracy in predicting specific xenobiotics in a sampling site where macroinvertebrates tolerant to 2.4-Dinitrophenol have been recorded.

As supported by numerous studies Polycyclic Aromatic Hydrocarbons (PAHs) are significant environmental pollutants that have the potential to accumulate in both marine and freshwater sediments. Bioaccumulated PAHs can expose additive, narcotic toxicity when organisms are exposed to them. Specific PAHs have been found to display significantly increased toxicity when exposed to ultraviolet (UV) light . Upon excitation by UV light, PAHs can generate singlet oxygen and reactive oxygen species **[18]**. These reactive compounds have the potential to cause damage to biological tissues **[19]**.

The toxicity or genotoxicity of Polycyclic Aromatic Hydrocarbons (PAHs), including the xenobiotic Fluoranthene, varies based on the number and arrangement of benzene rings, as well as the presence and location of substituents. These structural characteristics play a significant role in determining the specific biological effects exhibited by different PAH compounds **[20]**.

The available scientific evidence indicates that Fluoranthene is a PAH with phototoxic properties that can have negative effects on certain Oligochaeta species, highlighting the importance of monitoring and qualifying the exposure of these organisms to this chemical in order to protect their populations and maintain ecosystem health **[21]**.

The ANN model developed utilizing species tolerant to Fluoranthene as inputs and the corresponding measured concentrations of this xenobiotic as outputs, demonstrates a remarkably low Mean Squared Error (MSE) value of 0.0004. The MSE value serves as an indicator of the model's exceptional accuracy and precision in predicting Fluoranthene concentrations using input data derived from the tolerant species.  
When the model inputs are restricted solely to particular FS selected species belonging to the subclass Bivalvia, the MSE value significantly increases to 2.867 x 10-5 . This substantial increase in MSE suggests that these Bivalvia species are the most appropriate bioindicators of Fluoranthene, as their response to the xenobiotic leads to higher prediction errors compared to other species in the model.

Analyzing Bivalvia species presence, allochthonous species, including *Corbicula, Dreissena, Sinanodonta* and *Physella acuta* are generally known as species that exhibit higher pollutant tolerance compared to autochthonous native species like *Pisidium, Sphaerium, Unio* and *Theodoxus.* Therefore, the interpretation of the results should be guided by this fact **[22]**.The snail *Physella acuta* has achieved a global distribution and can now be observed as one of the most widely distributed recent snail species across the globe **[23,24]**. This species is recognized as a rapid (re)colonizer of freshwater systems with varying environmental conditions. Therefore, considering this characteristic, predictions based solely on the presence of this species as an indicator of xenobiotic presence should be confirmed using additional parameters **[25]**. Heavily modified and artificial aquatic habitats with high silting rates were found to be especially suitable for *S. woodiana* **[26]**.Numerous data clearly illustrate the vulnerability of oligochaetes to pesticides, and this finding is further supported by the implementation of an advanced Artificial Neural Network (ANN) model. Remarkably, the species *Nais barbata*, belonging to the Oligochaeta subclass, was absent from all sampling sites with herbicide Bromacil concentrations exceeding 0.04 mg/l, indicating its extreme sensitivity to this specific pesticide.

1. **Conclusion**  
   The developed Artificial Neural Network (ANN) models are well-performed in achieving high accuracy in predicting specific xenobiotics in river and serve as valuable tools for *in situ* prediction of river conditions and the rapid detection of elevated concentrations of particular xenobiotics, correlating with macroinvertebrate community structure. These models offer significant advantages, including their cost-effectiveness, environmentally friendly nature, and exceptional precision. They can perform as reliable guides in monitoring processes at eutrophic river sites. The high accuracy achieved by developed models make them a strong competitor to traditional chemical analysis methods able to serve as superior guide in monitoring and selection of river sites that need urgent intervention in aim to avoid higher exposure to xenobiotics and prevent possibly conducted consequences. Based on the findings indicating a significant increase in MSE, suggesting that Bivalvia species are the most suitable bioindicators of Fluoranthene due to their higher prediction errors compared to other species in the model, it can be specified that alien species (*Corbicula*, *Dreissena, Sinanodonta* and *Physella acuta)* generally exhibit greater tolerance to pollutants than native species (*Pisidium, Sphaerium, Unio, Theodoxus*).   
   The presence of certain native macroinvertebrate populations in locations where xenobiotics were detected does not establish a direct correlation between these species and serving as indicators for a specific xenobiotic. Their populations may be under certain levels of pressure in relation to the xenobiotic. Nevertheless, the ANN-based approach for estimation provides a brief guide for further exploration of specific river sites and assists to the responsible institutions to react promptly if necessary.

By strengthening data-driven approaches and advanced algorithms, these models can analyze various parameters, such as water quality indicators, habitat characteristics, and historical pollutant data, to identify sites where living organisms may be at greater risk. This proactive approach can help preserve the health and diversity of xenobitic sensitive species and also contribute to the overall protection and conservation of river ecosystems.

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