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RESEARCH ARTICLE

# A methodological approach for monitoring of riparian forests in protected areas on Danube islands

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## Abstract
In the last decades, invasive plant species have rapidly settled on the islands in the Danube River. This process is closely related to anthropogenic activities, such as felling and cultivation of soil for afforestation of Euro-American poplar trees throughout the Danube River basin. In this regard, it is important to: (i) determine the speed and scope of invasive vegetation establishment and (ii) determine more precisely the ongoing successional dynamics. Monitoring in permanent plots is needed to track the changes and assist in decision-making processes for the management of protected areas on the islands in the Danube River. The aim of the study is to describe and justify an innovative approach to analyse the dynamic of the invasion species occurrence and development as related to site conditions and management across the islands in the Danube River. The following methodological approach was considered: (1) selection of islands with well-preserved and sufficient in area covered by natural forest vegetation; (2) differentiation of strata by forest type, age and canopy cover; (3) setting up permanent sample plots for long-term monitoring in natural riparian forests.

## Keywords
Natural riparian forests, Danube River islands, monitoring, invasive species

## Introduction

In the last 50-60 years, invasive alien plant species (IAS) have rapidly settled on the islands in the Danube River. Their invasion was favoured by intensive anthropogenic activities such as felling and soil preparation for afforestation of Euro-American poplars throughout the Danube River basin. The opening of spaces usually leads to the establishment of invasive species on those territories, with species such as false indigo (*Amorpha fruticosa* L.), American ash (*Fraxinus americana* L.), Pennsylvanian ash (*Fraxinus penssylvanica* Marshall), box elder (*Acer negundo* L.), daisy fleabane (*Erigeron annus* (L.) Pers.) and others.

The presence of invasive alien plant species and the extent of their spread are crucial for the conservation of the autochthonous habitats, especially in vulnerable protected areas. Important tasks are the assessment of the speed and scale of the spread of IAS and the change in the course of natural regeneration processes. Monitoring is indispensable in order to observe these processes and to take important management decisions in protected areas and Natura 2000 sites in the Danube River. In the Republic of Bulgaria, the process of the most extensive designation of protected areas ended in 2007 (MOEW 2020). During the period 2011-2013, Bulgaria undertook the largest project for mapping and determining the conservation status of all habitats and species in the protected areas (MOEW 2013). In 2019, Bulgaria had to report to the European Commission on the status of habitats and species over the previous six years. During this time, it was necessary to monitor all habitats, including riparian forests (EEA 2020). In practice, however, no similar monitoring of the islands in the Danube River has been made. So far, no specific methodology has been developed to tackle this matter. The Republic of Bulgaria is unique in that it does not apply the National Forest Inventory. Despite the preparation of applicable methodology for the national forest inventory in Bulgaria (2017), it has not been adopted for various political reasons. In this respect, the lack of such information on what is happening in the Bulgarian forests, including those of riparian habitats, is quite tangible. In contrast to Bulgaria, a national forest inventory has been conducted in neighbouring Romania since 2007 (NIRDF 2020). The reporting of Romania to the European Commission was made for the period 2008-2012, yet there is no specific information available regarding the riparian forests of the islands in the Danube River.

Based on preliminary information from past studies (Hinkov et al., 2005, 2006, 2008, 2011; Donita et al., 2005, 2006, 2008; Munteanu et al., 2009), it has been suggested that the occurrence of invasive non-native plant species is significantly limited on islands with dense forest vegetation. This assumption is the basis of the initiation of joint research with Romania, as the islands are shared between the two countries alongside their Danube River border. In the spring of 2019, a project within the framework of the bilateral cooperation between the Bulgarian Academy of Sciences (BAS) and the Romanian Academy of Sciences (RAS) on “Invasive alien plant species in protected areas of the Danube Islands of Bulgaria and Romania” was launched. The planned studies are pilot and can be the basis of a broader study about the problem on both shores of the Danube River or in a consortium with other countries in the Danube River basin. At least four expeditions are scheduled within three years, which should take place on the islands in the Danube River, where natural riparian forests are preserved. Areas with dense forest vegetation will be explored. On the Bulgarian side with similar characteristics are the reserves of Milka, Kitka, Srebarna, the protected areas of "Stariya dab", "Kalimok-Brashlen", "Aleko-Telika", the islands Vetren (Ciocăneşti) and Aydemir (Chayka). On the Romanian side, these are the islands along 450 km on the Danube River course from Calafat to Călărași. Among the islands with the highest ecological diversity we can mention the following: Turcescu, Georgescu, Albina, Haralambie, Ciocăneşti (Vetren), Trămşani, Pisica, Şoimu, Turcescu, Cianu Nou and Fermecatu. In addition to the first two, all nine other Romanian islands are included in five Natura 2000 sites: – Olteniţa-Mostiştea Chiciu, Canaralele Dunării, Dunăre Olteniţa, Ciocăneşti Dunăre and Dunăre Ostroave.

**The purpose** of the study is to describe and justify an innovative approach to analyse the dynamic of the invasion species occurrence and development as related to site conditions and management across the islands in the Danube River.

***Objectives:***

To select suitable islands with compact complexes of natural forest vegetation.

To perform stratification according to habitat conditions, type of tree vegetation and cover of forest vegetation.

To establish permanent sample plots (PSPs) in order to test a methodological approach for long-term monitoring in natural riparian forests.

## Results

### Methodology of identification of suitable forests

In the summer of 2019, we began preparations of expeditionary studies and the establishment of permanent sample plots across the islands in the Danube River. It was necessary to select the appropriate islands, as well as to discover the approximate locations for the fieldwork. Forest management maps of Silistra (2011) and Călărași (2013), the Bulgaria platform of the WWF (Forests in Bulgaria, 2019), Google Earth Pro (2019) and the available topographic maps provided preliminary information for the implementation of expeditionary studies. The sites had to meet the following criteria: 1) the riparian forests located on the islands in the Danube River in Bulgaria and Romania to be natural; 2) the forest stands to be compact with an area of more than 20 ha; 3) the forests to be slightly influenced by logging or other economic activities in the last 30-40 years; 4) the forests to be located in protected areas or in NATURA 2000 sites.

The pre-selection was made on islands, which had been visited during previous expeditionary studies and which fully or partially had met the specified criteria. These are Golyama Barzina, Milka, Kitka, Vardim, Batin, Kalimok, Aleko, Pozharevo, Vetren (Ciocăneşti), Aydemir. The project between BAS and RAS (2019-2021) has been planned for a three- year period. In the first year, the two easternmost islands Vetren (Ciocăneşti) and Aydemir were visited. They fully meet the criteria mentioned above: both islands are in NATURA 2000 protected areas. They are one of the least affected by anthropogenic activity islands in the Danube River between Bulgaria and Romania. Both of them are disputed territories between the two countries. The island of Aydemir is divided between the two countries, while the island of Vetren (Ciocăneşti) is claimed by both countries at the same time. This has led to a lack of intensive economic activity over the last 40 years.

In the autumn of 2019, the first two expeditions were carried out to fulfil the set of tasks. This season was chosen because of the presence of fewer mosquitoes and ticks, existing canopy cover and the lack of flooded areas. In the autumn of 2019, the summer spectrum of vegetation was well preserved. The weather conditions had allowed identifying plant species that were also typical for the spring spectrum on the islands. On the two islands of Aydemir and Vetren (Ciocăneşti) the expeditions were organised on predetermined routes based on stratification. The island of Aydemir has an area of 77 ha (SFE Silistra, 2011), while the island of Vetren (Ciocăneşti) covers a total area of 280 ha (Amenajaemnt OS Călărași, 2013).

## Methodology of establishing permanent sample plots

***Stratification***. The purpose of strata is to identify forest areas that are homogeneous in terms of vegetation and structure. A major tool for differentiating strata was the publicly available free Google Earth Pro (2019) platform. It has options to view images that have been specifically made for these islands in 2019, 2018, 2017, 2014, 2013, 2011, during different seasons of the year. There were several photos taken in 2019 and 2018. There were also pictures of Vetren (Ciocăneşti) in 2004. Differences in structure and colour shades are crucial to the preliminary stratification of forest vegetation. Spring satellite images were the most appropriate, as they showed different colour of the crowns of the different tree species. In this way, the strata of white willow, white poplar, mixed natural forests, young willow forests and old poplar plantations were distinguished.

Based on images from Google Earth Pro (2019), four types of forest from natural riparian forests and one type of forest with semi-natural forests (old poplar plantations) had been previously identified on the two islands. Within the five expedition days, 16 permanent sample plots (PSPs) were established on a total forest area for the two islands of 357 ha.

The first distinct type of forest was that of "Young willow-poplar forests" in the early succession phase and with poorly developed soil substrate (sand and clay formations and undecomposed organic residue). Three PSPs were installed in this forest type.

The second type of forest was "Willow forests" (*Salix alba* x *fragilis*). It was among the most widespread on the two islands. Three PSPs were installed in this forest type.

The third type of forest, also widespread, was formed by "Natural mixed forests" composed of black poplar (*Populus nigra* L.), willow and white elm (*Ulmus laevis* Pall.). Seven PSPs were installed in this forest type.

The fourth type of forest was similar to the third one, however the tree floor was formed by a natural "White poplar natural forest" (*Populus alba* L.) with high density. Only one PSP was installed in this forest type.

The fifth type of forest was composed of "Old poplar plantations". They were much reduced and other tree species grew in the empty spaces. Two PSPs were installed there.

***Permanent sample plots***. The size of the permanent sample plots and the detailed measurement of the biometric indicators are in accordance with the methodology of the National Forest Inventory of Romania (NIRDF, 2019). This was determined by the lack of an established similar methodology in Bulgaria, the existence of such methodology already developed in Romania and the experience in forest inventory of the Romanian team.

Based on the type and age of the forest, circular sample plots were installed. In young forests, located in the "Pioneer Phase", PSPs were with a circular area of 200 $m^2$ (radius of the circle was 7.98 m). In mature forests that have reached the "Climax phase" or "Succession phase", PSPs were with a circular area of 500 $m^2$ (the radius of the circle was 12.62 m). The circular plots were delimited with a measuring tape or with VERTEX IV ultrasound hypsometer from Hagloff after a centre was selected. In the field, the PSP center was marked with oil paint. Usually a living tree had been chosen and it was considered the centre of the plot. Circular plots had several advantages over square or rectangular plots. Their installation and measurement were faster and more accurate. Using an ultrasound hypsometer and a precise compass were extremely convenient for this type of work. Finally, yet importantly, both teams from Bulgaria and Romania had been trained to work in a similar way. The border trees that were inside the plot have been marked also with oil paint. Two examples are given in tabular form, in which the methodology is indicated (from Table 1 to Table 8).

***Coordinates***. Coordinates were taken with a GPS device (Garmin GPSMAP64s). The location of each PSP was also marked with paint to allow future scientific observations.

**Table 1.** Positioning and tree parameters in PSP1

| № | Plant species | Azimuth (degrees) | DBH (cm) | H (cm) |
|---|---|---|---|---|
| 1 | *Populus hybridus* | 0 | 4.0 | 535 |
| 2 | *Populus hybridus* | 5 | 4.5 | 560 |
| 3 | *Salix alba (S. alba) X S. fragilis* | 105 | 4.5 | 578 |
| 4 | *S. alba* X S. *fragilis* | 108 | 5.0 | 650 |
| 5 | *S. alba* X S. *fragilis* | 110 | 5.5 | 670 |
| 6 | *S. alba* X S. *fragilis* | 115 | 6.0 | 653 |
| 7 | *S. alba* X S. *fragilis* | 116 | 5.0 | 560 |
| 8 | *S. alba* X S. *fragilis* | 117 | 4.5 | 560 |
| 9 | *S. alba* X S. *fragilis* | 119 | 5.5 | 657 |
| 10 | *S. alba* X S. *fragilis* | 120 | 7.0 | 680 |
| 11 | *S. alba* X S. *fragilis* | 125 | 5.0 | 605 |
| 12 | *S. alba* X S. *fragilis* | 126 | 4.5 | 600 |
| 13 | *S. alba* X S. *fragilis* | 129 | 5.0 | 600 |
| 14 | *Populus hybridus* | 152 | 1.5 | 155 |
| 15 | *Tamarix* sp. | 195 | | 200 |
| 16 | *Populus hybridus* | 205 | | 180 |
| 17 | *S. alba* X S. *fragilis* | 218 | 6.0 | 628 |
| 18 | *Populus hybridus* | 225 | | 170 |
| 19 | *Populus hybridus* | 228 | | 150 |
| 20 | *Populus hybridus* | 232 | | 190 |
| 21 | *Populus hybridus* | 265 | 2.0 | 416 |
| 22 | *Populus hybridus* | 282 | 4.0 | 532 |
| 23 | *Populus hybridus* | 290 | 3.0 | 500 |
| 24 | *Populus hybridus* | 300 | 4.0 | 530 |
| 25 | *Populus hybridus* | 308 | 5.0 | 535 |
| 26 | *S. alba* X S. *fragilis* | 310 | 5.0 | 543 |
| 27 | *S. alba* X S. *fragilis* | 310 | 5.0 | 505 |
| 28 | *S. alba* X S. *fragilis* | 312 | 8.0 | 710 |
| 29 | *S. alba* X S. *fragilis* | 313 | 5.0 | 500 |
| 30 | *S. alba* X S. *fragilis* | 315 | 7.0 | 700 |
| 31 | *Populus hybridus* | 335 | 4.0 | 532 |
| 32 | *Populus hybridus* | 359 | 4.0 | 537 |

**Table 2.** Dead wood in PSP1

| Dead wood | Lying down | | Standing | | |
|---|---|---|---|---|---|
| Diameter (cm) | Longitude (m) | Degree of rot | Diameter (cm) | Height (m) | Degree of rot |
| 15 | 3.0 | 1 | | | |

**Table 3.** Parameters of tree regeneration in PSP1

| Undergrowth | Plant species/Number of 40 m² | | | |
|---|---|---|---|---|
| h (cm) | *Populus hybridus* | *Fraxinus pennsylvanica* | *Morus alba* | *Amorpha fruticosa* |
| <10 | | | | |
| 10 to 30 | | | 1 | |
| 30 to 80 | | | | |
| 80-130 | | 1 | | 1 |
| above 130 | 3 | 1 | | |

**Table 4.** Phytocenological description in PSP1

| Species composition | Cover abundance (%) |
|---|---|
| Layer I | 45 |
| *Populus hybridus* (*P. deltoides* X P. *nigra*) | 20 |
| *Salix hybridus* (*S. alba* X S. *fragilis*) | 25 |
| Layer II | 20 |
| *Populus hybridus* | 10 |
| *Tamarix ramosissima* | 6 |
| *Salix hybridus* (*S. alba* X S. *fragilis*) | 4 |
| Layer III | 70 |
| *Xanthium italicum* | 40 |
| *Agrostis capillaris* | + |
| *Panicum sanguinale* | 3 |
| *Erigeron annuus* | 2 |
| *Populus hybridus* | + |
| *Echinochloa crus galli* | 7 |
| *Polypogon viridis* | |
| *Portulaca oleracea* | 2 |
| *Milium effusum* | 12 |
| *Chenopodium hybridum* | 2 |
| *Populus hybridus* | 1 |
| *Fraxinus pennsylvanica* | 1 |
| *Amorpha fruticosa* | + |

**Table 5.** Positioning and tree parameters in PSP7

| № | Plant species | Azimut (degrees) | DBH (cm) | H (cm) |
|---|---|---|---|---|
| 1 | *Ulmus laevis* | 0 | 34.0 | |
| 2 | *Fraxinus pennsylvanica* | 0 | 2.0 | 370 |
| 3 | *Fraxinus pennsylvanica* | 11 | 5.0 | 450 |
| 4 | *Fraxinus pennsylvanica* | 19 | 4.0 | 450 |
| 5 | *Fraxinus pennsylvanica* | 19 | 4.0 | 500 |
| 6 | *Fraxinus pennsylvanica* | 30 | 6.0 | 600 |
| 7 | *Ulmus laevis* | 47 | 45.0 | |
| 8 | *Fraxinus pennsylvanica* | 55 | 8.0 | 500 |
| 9 | *Populus nigra* | 77 | 45.0 | 2800 |
| 10 | *Ulmus laevis* | 85 | 4.0 | 400 |
| 11 | *Morus nigra* | 87 | 9.0 | 600 |
| 12 | *Populus nigra* | 110 | 86.0 | |
| 13 | *Ulmus laevis* | 115 | 28.0 | 1730 |
| 14 | *Populus nigra* | 117 | 95.0 | 3400 |
| 15 | *Ulmus laevis* | 134 | 40.0 | 2100 |
| 16 | *Populus nigra* | 165 | 47.0 | 3400 |
| 17 | *Populus nigra* | 185 | 75.0 | 3000 |
| 18 | *Populus nigra* | 210 | 81.0 | |
| 19 | *Ulmus laevis* | 220 | 30.0 | 1300 |
| 20 | *Ulmus laevis* | 280 | 45.0 | |
| 21 | *Populus nigra* | 310 | 105.0 | |
| 22 | *Ulmus laevis* | 330 | 28.0 | |
| 23 | *Fraxinus pennsylvanica* | 357 | 2.0 | 400 |
| 24 | *Fraxinus pennsylvanica* | 359 | 2.0 | 450 |

**Table 6.** Dead wood in PSP7

| Dead wood | Lying down | | Standing | | |
|---|---|---|---|---|---|
| Diameter (cm) | Longitude (m) | Degree of rot | Diameter (cm) | Height (m) | Degree of rot |
| 9 | 2.6 | 2 | 22 | 5.0 | 1 |
| 15 | 3.0 | 3 | 10 | 7.0 | 2 |
| 35 | 6.5 | 3 | | | |
| 30 | 3.0 | 5 | | | |
| 8 | 4.0 | 2 | | | |
| 8 | 3.5 | 2 | | | |
| 12 | 2.0 | 3 | | | |

| | | | | | |
|---|---|---|---|---|---|
| 6 | 3.5 | 2 | | | |
| 18 | 4.0 | 2 | | | |
| 9 | 2.0 | 2 | | | |
| 11 | 2.0 | 3 | | | |
| 12 | 7.0 | 4 | | | |

**Table 7.** Parameters of tree regeneration in PSP7

| **Undergrowth** | **Plant species/Number of 40 m²** | | | |
|---|---|---|---|---|
| h (cm) | *Ulmus laevis* | *Fraxinus pennsylvanica* | *Morus alba* | *Amorpha fruticosa* |
| <10 | | | | |
| 10 to 30 | | 4 | | 3 |
| 30 to 80 | 8 | 2 | | 4 |
| 80-130 | | 1 | | 2 |
| above 130 | | 2 | 1 | 4 |

**Table 8.** Phytocenological description in PSP7

| **Species composition** | **Cover abundance (%)** |
|---|---|
| Layer I | 70 |
| *Populus nigra* | 40 |
| *Ulmus laevis* | 30 |
| Layer II | 30 |
| *Ulmus laevis* | 15 |
| *Morus nigra* | 5 |
| *Amorpha fruticosa* | 10 |
| Layer III | 15 |
| *Fraxinus pennsylvanica* | 2 |
| *Calystegia sepium* | 1 |
| *Rubus caesius* | 2 |
| *Amorpha fruticosa* | 6 |
| *Periploca graeca* | 2 |
| *Ulmus laevis* | 2 |
| Layer IV | |
| *Asparagus* sp. | + |
| *Aristolochia clematitis* | + |

## Biometric measurements and parameters of the stand, the shrub floor and the ground grass floor

***Habitat conditions.*** An assessment of the habitat conditions (micro terrain forms) were made depending on the maximum water level marked on the stems of big trees. In this way, micro elevations (beams) with a flood height of less than 1 m, backwaters (flooded channels) with a flood height of more than 2 m and flat surfaces with a flood height between 1 and 2 m were established. Micro-terrain forms were a limiting factor for the spread of the vegetation type. For example, pure willow forests were found in floods above 2 m.

***Positioning.*** The positioning was performed by placing a tripod in the centre of the plot and a compass fixed on it. An azimuth position was determined for each tree in the plot. Positioning the trees in azimuth was fast and convenient. The trees were numbered, the record contains the type of plant species, diameter and height of the individual (Tables 1, 5). Re-measurement errors were almost non-existent. The positioning of the trees aimed to prepare a dossier of the available individuals, which would be compared in subsequent monitoring studies. For example, if a particular tree is not found on re-measurement, this will be taken into account in the new measurement due to its absence in the sample plot dossier.

***Tree diameters (DBH – diameter at 1.3 m).*** In the PSPs, all trees and shrubs with a DBH larger than 1 cm have been inventoried based on their azimuth in degrees from the centre of the PSP to the respective individual. DBH were measured with forestry callipers, and in the case of trees over 60 cm in DBH – with a tape to measure their circumference. The measurement of the diameter was necessary to determine the DBH structure of the forest, its stock and indirectly for estimating the age of the individuals.

***Height.*** The height of tall shrubs and short trees (up to 15 m in height) were measured with a pole (with an accuracy of 5 cm). Tall trees were measured with VERTEX IV with an accuracy of 25 cm. When measuring the heights of tall shrubs and small trees, the height-measuring pole was extremely effective, but it was heavy and inconvenient to carry. On the other hand, measuring heights with the ultrasound hypsometer was slower and required the involvement of another person to place the ultrasonic transponder. A big advantage was the small and compact size of this device. With it, of course, horizontal distances could be measured, which is extremely convenient in areas with a very dense undergrowth floor. Measuring the heights of the trees is necessary to determine the height of the tree floor, to establish the stem stock and forest growth.

***Wood stock.*** The calculation of the wood stock of living trees using assortment tables is a laborious process in a chamber setting. Moreover, the choice of the respective assortment table is not always sufficiently appropriate. The wood stock of live trees was calculated by using yield tables according to the Handbook of Dendrobiometry (1983) – for white willow (according to Nedyalkov, Tsakov, 1983), for black poplar (according to Nedyalkov et al., 1983), for white elm and Pennsylvania ash maple (according to Iliev, 1983). The measurement of the wood stock is directly related to the determination of the quantity and the percentage share of the tree species.

***Health condition.*** The health condition of the trees was evaluated using the following categories: healthy, damaged and dead. Establishing the health of trees is an indicator of the resilience and adaptability of the respective tree species.

***Dead wood.*** By measuring each average diameter and length of the section in the entire sample plots, the stock of the available dead wood – lying and standing, was established in cubic meters (Tables 2, 6). The degree of decomposition of dead wood was determined using a five-unit scale according to the methodological instructions of Zlatanov et al. (2013). Dead wood is an important parameter in assessing the conservation status of the forest. As already mentioned, most of the studied forests were entering a phase of old- growth forest. The large quantities of dead wood in a living forest were an indication that natural processes of waste and replacement of one stand with another are taking place. This was an indicator of the normal conservation status of the forest. On the other hand, the presence of a large amount of dead wood (for example in pure willow forests) was a clear sign of their health condition of the forest, even of its degradation and the beginning of a new succession. When describing the dead lying wood, a very serious challenge was identified and namely we recorded the presence of a dense cover of bur cucumber. It literally hid the available fallen trees and the probability of error increased.

***Age.*** Several trees in different PSPs were sampled with a Pressler increment borer to determine their age. This method is also time- consuming. It is recommended to take 1-2 samples of large trees that are at the same age. Most often, they have cavities inside and this increases the risk of jamming the drill. From younger trees with a diameter of 30-50 cm, it is necessary to take at least one sample of each tree species. These trees in most cases are of different ages with a difference of about 10-15 years. Determining the age of invasive tree species is a major task in the current research.

***Trees regeneration.*** The assessment indicators on trees regeneration with a height of up to 1.3 m were measured on a streak located along the diameter of the circle in a north-south direction, witюh a width of 3 m. For the undergrowth of tree and shrub species and available grass species (Tables 3, 7) were measured coverage, number by species composition, heights by height groups (Zlatanov et al., 2013). Working in a streak was preferred to circular measuring sites. This made taking photos easier and more informative. In the streak the work was faster, as well as the covered area was larger. The establishment of the indicated parameters for the assessment of the undergrowth gives an idea of the dynamics of the regeneration processes, respectively of the dynamics of the distribution of invasive tree species.

***Anthropogenic activity***. Anthropogenic activity was established in each sample plot and consisted of regulated felling, poaching violations, collection of dead wood, clearings or roads, artificial waste deposited by the river flow. An assessment of the conservation status and the degree of naturalness of plant layers was made. This allowed the extent of invasive species to be determined. The risk of the spread of invasive plant species was analysed on site using a scale with three levels (small, medium, large).

## Methodology of soil research

The methodological approach in soil research was focused on the surface layers of soils. This was due to the fact that the soils were young formations and were of the alluvial soil type (Fluvisols). They had been formed by the sediments of the river and had a formed complete genetic profile. In them the profile consisted of separate layers and had the type Ah, I, II, III, etc. On the periphery of the islands, there were alluvial sands, but towards the interior of the islands, under the influence of the forest vegetation, soil-forming processes and formation of a surface humus horizon were observed. The soil genetic profile consisted of separate layers that had been accumulated by the sediments of the river without a genetic link between them. Samples were taken from 0 to 20 cm from the soil. These samples were collected with a soil borer from each sample plot, in three replicates: on the concave, planar and convex part of the micro relief. These were mixed and an average sample for analysis was formed, labelled, packaged and transferred to the laboratory for analysis. The main soil characteristics that were analyzed were: total carbon according to Tyurin; total nitrogen – according to Keldal; mechanical composition – according to Kachinski (Donov et al., 1974). They were analysed according to the norms and scales adopted in Bulgaria. Establishing soil characteristics could provide information on differences in the accumulation of these substances under different forest types.

## Methodology the floristic investigation

The study of the floristic composition is a mandatory and fundamental part of the overall methodology for assessing the distribution of invasive native and alien species in the study area. The dynamics and richness of the flora are an indicator of the condition of the natural habitats and the biodiversity in them. In this case, the approaches to the field research and the scope of the in-house work are important. In this respect, the following criteria were leading in their selection:

1. Peculiarities of the study areas (they were relatively small in area, geographically isolated, low altitude, scarcely visited by humans and with conservation status).

2. Previous experience in floristic research of similar sites – freshwater island and riparian habitats.

3. Compatibility with other studies (stand assessment, soil, etc.).

4. Difficult access and limited time spent on the islands, which required both expediency and representativeness of the data on the studied flora.

***Methodological approach.*** The methodological approach followed the generally accepted practice in foreign and Bulgarian references (Apostolova-Stoyanova, Stoyanov, 2009; Dimitrov et al., 2012; Asenov, 2015; Glogov, Delkov, 2016, etc.). The field studies were performed using two methods: route method and the method of permanent sample plots. The routes were chosen to cover the most representative areas of the island with the most characteristic habitats and ecological niches.

***Sampling methodology.*** The sampling methodology covered the entire permanent sample area (200 $m^2$ or 500 $m^2$). Herbarium specimens were collected and deposited in some of the national herbariums.

***Analysis.*** The species composition established during the field research was analysed according to the following features: taxonomic, biomorphological, phytogeographical, ecological, taxa with conservation status, anthropization index, invasive alien species, economic benefits (honey, medicinal, etc.).

## Vegetation research methodology

While floristic research can capture the presence of an invasive alien species before its naturalization in a given area, phytocoenotic research provides information on the integration of populations of this species in local communities, successions and fluctuations in them. The study of vegetation is key when exploring the relationship between the different levels of biodiversity population-phytocoenosys-habitat-ecosystem.

The criteria for selecting a methodological approach for the phytosociological study as a part of the general methodology had some similarities with those adopted in the choice of approaches for the floristic research. The most important (for the first one) were the choice of the overall approach of the study – Dominant, Physiognomic or Floristic (Pavlov, 2006), their relevance, applicability, comprehensiveness and connection with the relevant syntaxonomic nomenclatures.

***Phytocenological description*** was performed according to the principles of the Western Phytocenological School of Braun-Blanquet (Braun-Blanquet, 1964; Westhoff, van der Maarel, 1973, etc.). The model of similar studies of vegetation in river habitats was followed (Pachedjieva, 2011; Tzonev, 2014; Gyosheva et al., 2015, etc.). The whole cycle of activities in conducting phytocenological analysis was divided into three main stages (Kent, Coker, 1992): field work, tabular (computer) processing of the collected data and syntaxonomic analysis.

***Sample plots.*** Sample plots standardised by size according to Chytry, Otypkova (2003) were set in each permanent sample plot: 16 $m^2$ for grass communities; 50 $m^2$ for shrub communities and 200 $m^2$ for forest communities (Tables 4, 8). The data from the phytocoenotic descriptions (relevés) made in them were also subjected to cluster analysis by the SYN-TAX program.

***Analysis***. An up-to-date classification of the vegetation in the studied area has been made, on the basis of which the classification of the habitats would be performed later. A community ordination (Q analysis) was performed using the Horn index and the principal components PCA method. Succession schemes were based on a comparative analysis of the floristic composition of the same territory (Mueller-Dombois, Ellenberg, 1974).

## Methodology for photographical data recording

Photos were taken with a small compact camera Sony Cyber Shot RX100 III, in the direction of the upper tree floor, intermediate shrub floor and lianas and the ground – grass floor. This was done from the centre of the plot in north and south directions. Diagnostic plants and fungal species were also photographed. Based on the experience of taking pictures in PSP, it is recommended to take photos from a precisely fixed place (shots from the centre and to the centre). Future research will also include the shooting of short films with a position from the PSP centre. The photos were in RAW format, which allows additional processing and offers higher image quality. All photos were numbered and archived. They have been intended as a source of information for future monitoring studies.

## Additional methodological approaches

***Database***. A database were developed in Excel, which contained the summarised tables and results. These are data available for the assessment for the habitat: soil conditions, micro relief, maximum water level, assessment data for stand (location of trees and shrubs, species composition, diameters, heights, health condition of trees, dead wood with estimated degrees of decomposition, stem volume of trees), biometric data on the ground floor – undergrowth, grass species and lianas, floristic and phytocenological data.

***Dossier***. A dossier was prepared for each PSP, which contained a general list of the plant species identified in the field (trees, shrubs, grass species and lianas). The spatial structure of the vegetation by layers was determined. The degree of invasiveness of the identified invasive alien plant species was assessed. The main threats and risks to ecosystems were identified.

# Conclusion

The proposed ideas for a methodological approach to monitor the spread of IAS in riparian forests on islands in the Danube River are applicable and can be included into methodologies of monitoring of forest habitats of the EEA in Bulgaria and in the protected areas Management Plans in Romania. It is important to pre-select the appropriate PSP sites based on the available data (e.g. management plans, maps, orthophotoplans, satellite imagery) and also to conduct field trips in the study areas, followed by a final choice and establishment of PSPs. The following principles have been applied in the development of the methodologies: (1) search for the most efficient and fast way of accessing information; (2) the most economical and feasible way to perform field and laboratory work; (3) creating a free access to the research.

The information obtained from these studies will be useful for updating existing data on invasive alien plant species and for future studies. Based on the collected field archive, guidelines and necessary measures for the management of invasive alien species will be provided.


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