Vegetation and Water of Some Spring-Wells of Po Valley (Northern Italy): Ecological Features and Management Proposals

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Abstract

Spring-wells (lowland springs, “fontanili”) are elements of Po Valley (Northern Italy) with ecological and historical importance: they provide water at a relatively constant temperature, generating unique ecosystems dependent on the groundwater outflow. Despite their importance, they are endangered by degradation processes as the expansion of urban areas and/or the intensification of agriculture, very marked in Po Valley. This research describes four spring-wells of Po Valley from a botanical and ecological perspective through phytosociological relevés and different ecological indexes. Water chemical-physical features are also considered (pH, temperature and ammonium ion, nitrite, nitrate, orthophosphate, chloride and organic matters contents). Plant communities of the spring-well beds shows a low number of species (5.8 ± 2.9) but also no exotic species while the vegetation of the banks has a high number of species (32.4 ± 9.8) but several of them are exotic/ornamental, with a low ecological value of the Ecological Index of Maturity (EIM = 4.4 ± 1.5) indicating disturbances, however moderate compared to the surrounding corn fields (EIM = 0.002 ± 0.001). All the water samples has high ammonium content (>0.50 ppm), the water of the spring-well B results the most polluted and both algae and *Callitriche obtusangula* (rare native species) grow largely in it, while spring-well C has less phosphates and more nitrite and is marked by the abundance of *Equisetum telmateia*. Exotic but historically important species as *Morus alba* were considered in the discussion, where management proposals to protect and enhance the studied spring-wells and others with similar characteristics are discussed.

Introduction

Wetlands play an important role in providing ecosystem goods and services to human society. However, the neglect of their importance intensified their loss due to the widespread conversion of wetlands because of shortages of land that is suitable for agriculture and urban settlement (He et al, 2015). They are very often environments rich in species, local climate regulators as well as elements that enrich the landscape and the historical-cultural heritage of the territory in which they are located (Ramsar Convention Bureau 2001; Mitra et al. 2003). Although they provide important services, wetlands are declining faster than any other ecosystems (MEA 2005) and this phenomenon affects the whole Globe, in particular those territories where the expansion of urban areas and agricultural activities is more marked (Hu et al. 2017). In fact, recent studies estimated that at least 33% of global wetlands has been lost since 2009 (Hu et al. 2017) and 64-71% since the early 1900 (Davidson 2014), and that this loss is mainly due to land conversion as well as to climate change (Lin and Yu 2018).

The Po Valley (or Po Plain) is the largest plain in Italy and in southern Europe (47.800 km²). It is bordered to the north and west from the Alps, to the south from the Apennines and to the east from the Adriatic Sea. Today much of the territory of Po Valley is farmed according to intensive farming methods and occupied by large, urbanized areas that grew rapidly during the last century and are still in expansion. Considering the geological, pedological and environmental characteristics, Po Valley is divided into two main areas: the “high plain” and the “low plain” (Martinis et al. 1976; Andreis 2002). The “high plain” is at
the base of the slopes of the Alps and the Apennines (over 100-150 m a.s.l.) and is characterized by very permeable soils whose particles size consists mainly of coarse elements (stones, gravel, and sand). In the “high plain”, rainwater penetrates the soil in depth until it reaches waterproof rocks/sediments on which it flows and then resurfaces at lower altitudes (100-80 m a.s.l.), giving rise to resurgences (Toniolo 1933; Minelli et al. 2002; De Luca et al. 2014). The resurgences are located along a large part of Po Valley forming a belt from west to east parallel to the foothills (about 800 km long and 25 km wide) which is known as the “resurgence belt” and which delimits the “high plain” from the “low plain” (Martinis et al. 1976; De Luca et al. 2014). The soils of the “low plain” have finer and impermeable particles (silt and clay) that allow the water to stagnate and generate swamps (covered by hygrophilous forests) which, however, were reclaimed by man (from the Roman age up to the last century) to promote agriculture (Frattini 2008; De Luca et al. 2014). Human work transformed the resurgences of Po Valley into spring-wells (lowland springs, “fontanili”) (Corbetta 1969; De Luca et al. 2014), concentrating their points of emergence. The spring-wells were obtained by enlarging the natural valleys in the land, encouraging the water to surface, forming a more or less rounded “head”, that can collect more springs. The water collected is channelled into a canal or “central axis” of the watercourse (Corbetta 1969; De Luca et al. 2014).

The spring-wells are groundwater-dependent ecosystems (GDEs) defined as “ecosystems for which current composition, structure and function are reliant on a supply of groundwater” (Kløve et al. 2011). Among GDEs, spring-wells are unique ecosystems dependent on the groundwater outflow (EC 2015). The spring-wells provide water with relatively constant temperature (11-13°C) (Cavagnis and Orsini 1992): seasonal variations affect the water temperature with a delay of 2-4 months, making the coldest period in April and the warmest in October, creating particular environments where rare plants and animals of community interest can live (Bertolani Marchetti 1959; Pisoni and Valle 1992; Viaroli et al. 2003; De Luca et al. 2005; Frattini 2008; Alessandrinì et al. 2011; Bischetti et al. 2012). For this reason, the spring-wells are included in the Habitat Directive (EC 1992) but most of them are currently at risk due to degradation processes happened mainly in the Sixties (Bischetti et al. 2012).

A large part of Po Valley falls within the Lombardy region which is one of the largest (23.800 km²), most anthropized (10 million people) and productive regions of Italy and of the Alpine macro-Region (EUSALP). In the plains of Lombardy there are few spring-wells that were not destroyed by the expansion of urban areas and/or intensification of agricultural practices. Only for a few of these scientific studies relating to their biotic and abiotic components of the ecosystem were carried out (Piazzoli Perroni 1959; Albergoni et al. 1977; Bertuletti 1992; Cavagnis and Orsini 1992; Pisoni and Valle 1992; D'Auria and Zavagno 2005; Frattini 2008; Balestrini et al. 2016, 2021). In 2018 the ANBI Association (Associazione Nazionale Bonifiche, Irrigazioni e Miglioramento Fondiario, https://www.anbi.it) and Lombardy Region financed the “AcquaPluSS” project (https://www.anbilombardia.it/portfolio-items/progetto-acquapluss/) to preserve/improve and enhance the spring-wells of Lombardy. This project proposes a series of activities/work to be carried out in some areas of Po Valley including study, enhancement, and restoration
of four spring-wells located in Brescia province (Lombardy) in the territory managed by the Oglio Mella Reclamation Consortium.

This research is part of the “AcquaPluSS” project and aims at analysing the vegetation (from a floristic and ecological point of view) of four spring-wells in the plain of the province of Brescia. In particular, phytosociological relevés of the vegetation of the “heads” of the spring-wells were carried out and a system of ecological indices (Taffetani and Rismondo 2009; Rismondo et al. 2011; Giupponi et al. 2015) was applied in order to highlight elements of biological value and evaluate the state of conservation/degradation of these environments. Furthermore, the chemical-physical characteristics of the waters from the spring-wells were analysed to assess their level of pollution and highlight any relationships with the presence/absence of hygrophilous plants. The results of the vegetation and water analyses were finally processed to provide good practices to the Oglio Mella Reclamation Consortium (and other territorial managers of environments with similar characteristics) useful to preserve, restore and enhance the vegetation of these wetlands.

**Materials And Methods**

**Study areas**

The four springs considered in this research are in Po Valley within the province of Brescia (Lombardy region, Italy) (Table 1, Fig. 1). Some of them (A and C) are surrounded by maize fields (corn cultivated according to intensive farming techniques) while others (B and D) are in urban areas and are adjacent to houses/gardens and/or roads. At the edge of some spring-wells there are recreational areas where various exotic/ornamental species were planted such as: *Eryobotrya japonica*, *Aesculus hippocastanum*, *Hibiscus* spp., *Rosa* spp., *Musa* spp. Since 2012 the ordinary management work (cleaning the spring-well riverbed, cutting dangerous trees, etc.) of this spring-wells has been carried out by the Oglio Mella Reclamation Consortium.

**Table 1**

| Spring-well identification code | Municipality     | Latitude N       | Longitude E      | Elevation (m a.s.l.) |
|-------------------------------|------------------|------------------|------------------|----------------------|
| A                             | Castel Mella (BS)| 45°29’23.9”     | 10°08’03.8”      | 100                  |
| B                             | Brandico (BS)    | 45°27’09.2”     | 10°03’03.9”      | 96                   |
| C                             | Lograto (BS)     | 45°28’39.8”     | 10°03’41.7”      | 106                  |
| D                             | Trenzano (BS)    | 45°28’41.2”     | 10°01’15.1”      | 104                  |

The study area is enclosed in the temperate continental bioclimatic zone (Rivas-Martínez et al. 2004) and in the Po Plain ecoregional section (Po Plain province, Temperate division) (Blasi et al. 2014). This
ecoregional section is 49,800 km² wide, and it is constituted by Po Plain territories with similar climatic features, physiography (litho-morphology is characterised by the clastic plain of the Po Plain foredeep) and potential natural vegetation (plain forests of *Quercus robur* and *Carpinus betulus* and riparian forests close to the streams/rivers) (Del Favero 2002; Verde et al. 2010; Blasi et al. 2014). It includes a considerable amount of agricultural areas (81%) with extensive artificial surfaces (10%) and few natural and semi-natural areas (6%) (Blasi et al. 2014). Annual precipitation ranges from 580 mm up to 1,350 mm (with winter minimum) while the mean annual temperature ranges between 11°C and 14°C.

**Vegetation analysis**

Data on the current vegetation of the four spring-wells were collected by performing 20 phytosociological relevés according to the method of Braun-Blanquet (1964) and using his conventional abundance/dominance scale (*r*, rare species in the relevé; +, coverage < 1%; 1, coverage 1–5%; 2, coverage 5–25%; 3, coverage 25–50%; 4, coverage 50–75%; 5, coverage 75–100%). In detail, 5 relevés for each spring-well “head” were performed: one in the bed of spring-well (zone I) and four in the banks/edges (zone II) (Fig. 1). In addition to the relevés of the spring-well, 5 phytosociological relevés were carried out in the maize fields adjacent to the spring-well A and C in order to compare the vegetation of the spring-well and of the most common farming system of Po Valley.

The relevés were performed during summer months (June–July) of 2020 and an area of 30 m² was studied for the vegetation of the banks/edges and that of the bed of spring-well, while for the maize fields an area of 25 m² (5 × 5 m) was examined. Plant species (phanerophytes) were identified using the keys of Pignatti (2017). The abundance (rarity) in Lombardy of each species found in the spring-wells was defined following Martini et al. (2012) while life forms according to Raunkiaer’s (1934) categories follow Pignatti (2017).

The data of the relevés were arranged in a matrix (relevés x species) where abundance/dominance indices of Braun-Blanquet were converted into the percentage of coverage in accordance with Canullo et al. (2012) (*r*, 0.01%; +, 0.5%; 1, 3.0%; 2, 15.0%; 3, 37.5%; 4, 62.5%; 5, 87.5%) to perform statistical analysis. A hierarchical cluster analysis and detrended correspondence analysis (DCA) were performed to identify floristic similarities/differences among the relevés. Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic mean method (UPGMA) and the chord distance coefficient (Legendre and Gallagher 2001). The optimal number of clusters (the smallest number of clusters which account for the largest amount of variation in the data) was assessed based on the “elbow” method that consists of plotting the explained variation as a function of the number of clusters and picking the elbow of the curve as the number of clusters to use. Statistical analysis (cluster analysis and DCA) was performed using R 3.6.1 software (R Development Core Team 2015).

The ecological index of maturity (EIM) (Giupponi et al. 2015) was calculated for each type of vegetation returned by cluster analysis. The EIM measures the level of disturbance affecting a plant community considering phytosociological class, chorotype, and coverage of each species present, and was calculated according to the following formula (Giupponi et al. 2015, 2017a):
where IM is the index of maturity, IE is the index of the exotic component and IL is the index of the endemic component. EIM values can range from 0 (high vegetation disturbance) to 9 (undisturbed vegetation).

The IM provides a measure of the actual stage of maturity of a plant community and is calculated as follows Taffetani and Rismondo (2009):

\[
\text{EIM} = \frac{\text{IM} \left[ (1 - \frac{\text{IE}}{100}) + \frac{\text{IL}}{100} \right]}{1 + \frac{\text{IL}}{100}}
\]

where IM is the index of maturity, IE is the index of the exotic component and IL is the index of the endemic component. EIM values can range from 0 (high vegetation disturbance) to 9 (undisturbed vegetation).

The IM provides a measure of the actual stage of maturity of a plant community and is calculated as follows Taffetani and Rismondo (2009):

\[
\text{IM} = \frac{\sum_{i=1}^{n} (c_i m)}{C}
\]

where \(c_i\) is the coverage value of each single species, \(i (i = 1, 2, ..., n)\) is the number of species, \(m\) is the coefficient of maturity of the phytosociological class to which each species belongs, \(C\) is the total coverage value obtained by summing the values of \(c\) for all the species present. The coefficient of maturity \((m)\) is the value assigned to the main phytosociological classes of European vegetation according to the physiognomic-structural, synecological characteristics and the syndynamic role of the vegetation of each class (Taffetani and Rismondo (2009); Rismondo et al. (2011); Giupponi et al. 2017a) (Table 2).
Table 2
Coefficient of maturity \((m)\) of each phytosociological class of European vegetation. The names of the syntaxa are in accordance with Biondi et al. (2014). *, cultivated or exotic species that have not evolutionary significance (not attributable to specific phytosociological class)

| Phytosociological class                                                                 | \(m\) |
|----------------------------------------------------------------------------------------|------|
| * cultivated or exotic species                                                         | 0    |
| STELLARIETEA MEDIAE                                                                   | 1    |
| ORYZETEA SATIVAE                                                                     | 1    |
| POLYGONO ARENASTRI-POETEA ANNuae                                                      | 2    |
| CHARETEA FRAGILIS                                                                     | 2    |
| LEMNETEA MINORIS                                                                     | 2    |
| POTAMETEA PECTINATI                                                                   | 2    |
| TUBERARIETEA GUTTATAE                                                                 | 2    |
| CAKILETEA MARITIMAE                                                                  | 2    |
| SAGINETEA MARITIMAE                                                                  | 2    |
| THERO-SUAEDETEA SPLENDENTIS                                                           | 2    |
| ARTEMISIETEA VULGARIS                                                                | 3    |
| MOLINIO-ARRHENATHERETEA                                                              | 4    |
| BIDENTETEA TRIPARTITAE                                                               | 4    |
| ISOETO-NANOJUNCETEA                                                                  | 4    |
| FESTUCO VALESIAE-BROMETEA ERCTI                                                      | 5    |
| KOELERIO GLAUCAE-CORYNEPHORETEA CANESCENTIS                                         | 5    |
| LYCEO SPARTI-STIPETEA TENACISSIMAE                                                   | 5    |
| NARDETEA STRICTAE                                                                   | 5    |
| POETEA BULBOSAE                                                                     | 5    |
| ASPLENIETEA TRICHOMANIS                                                              | 5    |
| FESTUCO-SESLERIETEA                                                                 | 5    |
| CARICETEA CURVULAE                                                                  | 5    |
| SEDO ALBI-SCLERANTHETEA BIENNIS                                                      | 5    |
| THLASPIETEA ROTUNDIFOLII                                                             | 5    |
| Phytosociological class                                          | m   |
|---------------------------------------------------------------|-----|
| AMMOPHILETEA                                                  | 5   |
| CRITHMO MARITIMI-STATICETEA                                   | 5   |
| JUNCETEA MARITIMI                                             | 5   |
| PEGANO HARMALAE-SALSOLETEA VERMICULATAE                       | 5   |
| SARCOcornietea Fruticosae                                     | 5   |
| SPARTINETEA GLABRAE                                           | 5   |
| CARDAMINETEA HIRSUTAE                                         | 6   |
| GALIO APARINES-URTICETEA DIOICAE                              | 6   |
| PARIETARIETEA JUDAICAE                                        | 6   |
| ADIANTETEA CAPILLI-VENERIS                                    | 6   |
| LITTORELLETEA UNIFLORAE                                       | 6   |
| MONTIO FONTANAE-CARDAMINETEA AMARAE                           | 6   |
| OXYCOCCO PALUSTRIS-SPHAGNETEA MAGELLANICI                     | 6   |
| PHRAGMITO AUSTRALIS-MAGNOCARICETEA ELATAE                     | 6   |
| SCHEUCHZERIO PALUSTRIS-CARICETEA NIGRAE                       | 6   |
| EPILOBIETEA ANGUSTIFOLII                                      | 7   |
| MULGEDIO ALPINI-ACONITETEA VARIEGATI                          | 7   |
| TRIFOLIO MEDII-GERANIETEA SANGUINEI                           | 7   |
| CALLUNO VULGARIS-ULICETEA                                     | 8   |
| CISTO LADANIFERI-LAVANDULETIA STOECHADIS                      | 8   |
| CISTO CRETICI-MICROMERIETEA JULIANAE                          | 8   |
| CYTISETEA SCOPARIO-STRIATI                                    | 8   |
| RHAMNO CATHARTICAE-PRUNETEA SPINOSAE                           | 8   |
| ROSMARINETEA OFFICINALIS                                      | 8   |
| NERIO OLEANDRI-TAMARICETEA AFRICANAE                          | 8   |
| ERICO CARNEAE-PINETEA SYLVESTR                               | 9   |
| JUNIPERO SABINAE-PINETEA SYLVESTR                            | 9   |
| QUERCO ROBORIS-FAGETEA SYLVATICAE                             | 9   |
The IE provides the percentage of exotic species of a plant community considering exotic species coverage compared to total coverage while IL gives the percentage of endemic species (Giupponi et al. 2015). The IE measures the degree of exotic contamination and artificiality of the vegetation in relation to human pressure. National and European bibliographic sources were consulted for the attribution of phytosociological class to each species (Mucina et al. 1993; Del Favero 2002; Ubaldi 2008a, b; Landolt et al. 2010; Biondi and Blasi 2015). Syntaxonomic nomenclature follows that of the Vegetation Prodrome of Italy (Biondi et al. 2014; Biondi and Blasi 2015).

### Water analysis

Three water samples of 1 L were collected in each spring-wells in three different months: May, July and September 2020. The water samples were collected at the “head” of the spring-wells after measuring the water temperature with a digital thermometer (Checktemp® 1 - HI98509 Hanna Instruments). The 12 water samples were stored in dark bottles placed in the laboratory at a temperature of 4°C until their chemical analysis. The ammonium ion, nitrite, nitrate, orthophosphate and chloride contents were evaluated for each water sample using a spectrophotometric method. In detail, the ammonium ion content in the water was assessed using Nessler’s reagent while the nitrite was quantified using the Griess reagent (CNR-IRSA 2003; Kruse and Mellon 1953). The nitrate content of each water sample was obtained according to Tartari and Mosello (1997) while the water-soluble orthophosphate ion was determined in accordance with Menzel and Corwin (1965). The determination of chloride was carried out by the Mohr method (Vollenweider 1962). A digital pH meter (BASIC, Denver Instrument) was used to measure the pH of each water sample and the concentration of oxygen in the water (indicator of organic matters) was determined using permanganate value method (Kubel method) (Heukelekian et al. 1954). Each chemical analysis was performed in triplicate.

The results of the chemical-physical analyses of the water and the data of the relevés carried out in the riverbeds of the spring-wells were analysed used canonical correspondence analysis (CCA) to highlight the most important variables that differentiate the spring-wells and the presence/absence of species. CCA was performed using “vegan” package of R (R Development Core Team 2015).

### Results

#### Flora and vegetation
During the study 141 species were identified (Table S1). Most of them (73%) are perennial terrestrial plants (hemicriptophytes, phanerophytes, geophytes and nano-phanerophytes) while the therophytes (annual/ephemeral plants) are 26% of the biological spectrum (Fig. 2). The latter are mostly species of Stellarietea mediae phytosociological class, typical of urban/anthropic environments (such as: *Anisantha sterilis, Hordeum murinum, Lamium purpureum* and *Avena fatua*). The aquatic plants (hydrophytes), that are mainly located along the bed of the spring-wells, make up 1% of the flora list. 20% of the identified species are exotic (Fig. 2). Most of these are ornamental/garden plants not very common or rare in the natural environments of Lombardy (*Nandina domestica, Spirea chamaedryfolia, Iris orientalis, Oxalis articulata* etc.) but there are also naturalized and invasive species very common in northern Italy such as *Robinia pseudoacacia, Morus alba, Ailanthus altissima, Ambrosia artemisiifolia* and *Erigeron canadensis*. No endemic species were identified while some uncommon or rare native species of Lombardy were found, such as *Callitriche obtusangula* (aquatic species presents in A, B and D spring-wells) (Fig. 2), *Prunus domestica, Carex riparia, Poa palustris, Lemna minor* and *Helosciadium nodiflorum*.

The dendrogram resulting from the cluster analysis (Fig. 3) shows three groups (clusters) of relevés corresponding to the three different vegetation types present in the three zones were the relevés were carried out:

- **I**, hydrophilic vegetation of the bed of spring-wells: characterized by aquatic/helophytic species of *Lemnetea monoris, Potametea pectinati* and *Phragmito australis-Magnocaricetea elatae* phytosociological classes (Table S1);

- **II**, vegetation of the banks/edges: characterized by ruderal species of *Stellarietea mediae* and *Artemisietea vulgaris*, and species of shrublands (*Rhamno catharticae-Prunetea spinosae* and *Robinietea*), meadows (*Molinio-Arrhenatheretea*), woods (*Querco roboris-Fagetea sylvaticae* and *Salici purpureae-Populetea nigrae*) and marsh/lacustrine environments (*Phragmito australis-Magnocaricetea elatae*) (Table S1);

- **III**, vegetation of maize fields: characterized by *Zea mays* and some cosmopolitan weeds including *Cynodon dactylon* and *Sorghum halepense* (Table S1).

DCA biplot (Fig. 3) confirms the results of the cluster analysis that does not show floristic differences among the banks/edges vegetation of the four spring-wells.

Figure 4 shows the ecological characteristics of the three types of vegetation. The plant community of the spring-well bed (I) has a low number of species but has no exotic species, so the values of IM and EIM are the same (Giupponi et al. 2015). The vegetation of the banks, instead, has a high number of species but several of them are exotic/ornamental. The EIM value of vegetation II is lower than that of IM and this indicates disturbances affecting this plant community. However, these disturbances are moderately low if compared to those affecting corn fields (III) where there is a dominant exotic species (*Zea mays*) and ruderal weeds able to survive in disturbed and/or degraded environments.

Water
Table 3 shows the chemical-physical features of the spring-wells water. All the water samples have high (and similar) ammonium content, over the legal quality thresholds for groundwater. The water of the spring-well B results the most polluted. In fact, it has high concentration of orthophosphate and organic substance (concentration of oxygen due to the presence of organic oxidisable substance) in addition to the high concentration of ammonium. Moreover, the water of B has a higher concentration of nitrate but within the legal limit and coherent with the results in Balestrini et al. (2021). The oxygen concentration of water in spring-well B was over the threshold only in May (5.37 ppm) while the water of the spring-well C exceeded the threshold for nitrite only in September (0.70 ppm) and the water of the spring-well A had exceeded the threshold for orthophosphate only in July (1.18 ppm). The chloride and nitrate values of all spring-wells are below the quality thresholds for groundwater.

Table 3 chemical-physical characteristics of the water of the four spring-wells (A, B, C and D) compared with the legal thresholds of the quality of groundwater (Law 152/2006). The thresholds for surface water to produce drinking water (Law 152/1999) were used for pH ad orthophosphate. Key: SE, standard error; *, value over the limit only once (in May or July or September 2020); **, value over the limit for more than one sampling period

| Parameter          | A          | B          | C          | D          | Threshold value |
|--------------------|------------|------------|------------|------------|-----------------|
| Temperature (°C)   | 16.30 0.36 | 15.80 0.87 | 15.87 0.31 | 16.67 0.31 |                 |
| pH                 | 7.21 0.05  | 7.20 0.05  | 7.23 0.00  | 7.35 0.09  | 6.5 - 9.5       |
| NH$_4^+$ (ppm)     | 0.56** 0.00| 0.54** 0.01| 0.57** 0.00| 0.55** 0.01| 0.50            |
| NO$_3^-$ (ppm)     | 2.24 1.88  | 5.13 1.88  | 0.58 0.45  | 1.54 0.57  | 50.00           |
| NO$_2^-$ (ppm)     | 0.05 0.03  | 0.05 0.03  | 0.29* 0.36 | 0.01 0.00  | 0.50            |
| PO$_4^{3-}$ (ppm)  | 0.65* 0.46 | 0.75** 0.15| 0.35 0.09  | 0.42 0.11  | 0.70            |
| Cl$^-$ (ppm)       | 25.41 1.02 | 20.09 1.10 | 21.27 1.06 | 17.14 1.07 | 250.00          |
| Kubel oxidation (O$_2$ ppm) | 1.94 0.92 | 2.21* 2.73 | 0.67 0.14  | 0.52 0.05  | 5.00            |

The results returned by CCA are shown in Fig. 5. The biplot shows a main ecological gradient along the first axis (CCA1) which explains most of the variance in the dataset. In particular, along the CCA1 ammonium and nitrite in water decrease while nitrate, orthophosphate and organic substance increase. The water of spring-well B is the most polluted and both algae (indicators of eutrophic water) and Callitriche obtusangula are abundantly present in it (Table S1). The same plants also grow in the bed of A and D spring-wells but with less coverage values (Table S1). Spring-well C differs from the others for water with less phosphates and more nitrite, moreover in its bed (and on its banks) Equisetum telmateia is abundant (Fig. 5, Table S1). Helosciadium nodiflorum is also abundant in C but is present, with lesser coverage, also in A and D while it is absent in B. The spring-well A is characterized by some hygrophilous species that are little or not present in the other study sites, such as: Poa palustris, Hypericum tetrapertenum, Phalaroides arundinacea and Lythrum salicaria. Agrostis stolonifera is the only species
found in all the beds of the spring-wells considered in this research. Water pH values of the considered spring-wells are very similar and coherent with the values of other spring-wells in Po Valley measured by Cavagnis and Orsini (1992) and Balestrini et al. (2021) while temperature measured where the water springs resulted slightly higher (16.16 ± 0.41°C) comparing other values in previous research (Cavagnis and Orsini 1992). Also, the chloride content, also if far below the threshold, (250 ppm), resulted slightly higher (Table 3) comparing with values reported in other studies (De Luca 2014; Balestrini et al. 2021).

Discussion

Vegetation and water

The floristic-vegetational results of the four spring-wells show signs of environmental degradation but also valuable elements that are common to all four study areas at various level. The high number and/or coverage of ruderal therophytes of *Stellarietea mediae* class living in the spring-wells banks/edges, and even more in the corn fields, is a clear sign of disturbance as suggested by the low EIM value (far lower than the maximum value of 9 related to a mature wood free from exotic species) (Fig. 4). Such disturbance is caused by human activities, more precisely it is due to the intensive management of the surrounding fields and to the presence of urban areas around the considered spring-wells. These wetlands are deeply influenced by the adjacent highly disturbed areas as corn fields where IM and EIM values are extremely low (Fig. 4). Similar values were found for other corn fields of Po Valley (Giupponi et al. 2013) and are due to the presence of annual/short lived species (mainly cosmopolitan or exotic species) of the initial stages of the plant succession. These species can reach the banks because spring-wells are patchy and narrow (not extended in width) in the agricultural and urban landscape that characterizes a great part of Po Valley (Bischetti et al. 2012). The low EIM value of banks vegetation is also determined by the presence of the most diffused trees naturalized in Po Valley, especially on the edges of fields and roads and in little wooded spots as *Robinia pseudoacacia* and *Ailanthus altissima* (Del Favero 2002; Celesti-Grapow et al. 2010). White mulberry (*Morus alba*) can be added to these. Even though being an exotic plant, it can be considered an added value if present in these environments. White mulberry is in fact a *Moraceae* native to Eastern Asia probably introduced in Europe in the XII century (archeophyte) for silkworm farming (*Bombyx mori*) and grown in Italy from the XV Century (Bof 2017). At the end of the Nineteenth century Italy was the main producer of silk in Europe (Bof 2017), in Brescia, for example, during 1825, 26.800 tons of cocoons were produced (Fappani and Maffeis 1994; Bof 2017). Then, from the beginning of the Twentieth century, when synthetic textile fibres were introduced and importing silk from China became cheaper, the silkworms breeding in Italy and consequently the cultivation of the white mulberry narrowed gradually and almost totally disappeared in many Italian areas (Bof 2017). The presence of mulberry in all the spring-wells under study is therefore an element of historical and cultural value as a memory of agricultural activities, landscapes and agro-ecosystems of Po Valley almost totally disappeared today. IM and EIM, as formulated by their authors (Taffetani and Rismondo 2009; Rismondo et al. 2011; Giupponi et al. 2015), do not consider the historical-cultural value that some exotic species (as white mulberry) can have if present in determined environments (agro-
ecosystems). The limit of these indexes is to consider every exotic species as a degradation and disturbance element, aspect that could be overcome integrating these indexes or creating new ones able to consider the historical-cultural value of these kind of exotic species in the plant communities where they are present.

Beyond the ruderal spontaneous species and the exotic invasive/naturalized species that naturally colonized the banks of the spring-wells, ornamental (exotic) species planted out of controls by local inhabitants (oral communication) were detected. These species, although not invasive, contribute to the degradation of the spring-wells either from an ecological (they lower EIM values) and physiognomic/landscaping point of view and their presence suggests the necessity of involving the local community in outreach/formative activities (Schild 2016). This would empower people awareness on the value and biological, scenic, and cultural importance of spring-wells and to know the basic concepts of nature conservation.

Together with the ruderal and exotic species, the floristic analysis allowed the identification of native species typical of Po Valley wetlands, as the ones of *Lemnetea minoris, Potametea pectinatii, Phragmito australis-Magnocaricetea elatae* and *Salici purpureae-Populetea nigrae* phytosociological classes (Table S1). These species present in areas I and II, represent the biological-naturalistic valuable element of the spring-well analysed. This applies particularly for spring-well A that is the richest in wetland species some of which found exclusively (such as: *Carex riparia* and *Lythrum salicaria*) and/or that have noteworthy coverage (such as: *Poa palustris, Phalaroides arundinacea* and *Populus nigra*) on the banks/edges of this specific spring-well. Spring-well A could be then employed as “source” area where to collect plant material (seeds or other propagation organs) to employ in possible re-introduction/re-population or ecological restoration initiatives of this or nearby spring-wells banks/edges (“sink” areas).

The riverbed of the spring-wells (zone I), although it has much less species comparing to banks/edges, does not present exotic species, and in fact its EIM and IM values are higher comparing to the ones of banks/edges vegetation (Fig. 4). Such value is however far from the maximum EIM value (9) for the reason that in zone I, a series of biotic and abiotic disturbances more or less recent (among which the constant presence of running water and the excavation and cleaning initiatives of the riverbed and spring-wells) hamper the establishment of a soil layer able to host species of the mature forest typical of Po Valley. In zone I, additionally to the absence of exotic species, there are some species uncommon/rare in Lombardy and in general in Italy as *Callitriche obtusangula*. From the analysis of the chemical-physical features of spring-wells (Table 3) and from CCA (Fig. 5) it might seem that *Callitriche obtusangula* needs eutrophic waters with a high nitrate, phosphates, and organic substance concentration as in spring-well B in which *Callitriche obtusangula* has interesting values of coverage and algae are abundant (Table S1; Fig. 1). Conversely, in waters poorer in phosphates, nitrate and organic substance, *Callitriche obtusangula* is less abundant (as well as algae) till being totally absent in spring-well C, that is the one with the least eutrophic water and that presents the minor number of species (3) in the riverbed (Table S1). The presence of *Callitriche obtusangula* in polluted waters is explained by being a nitrophilous and salt dependent species (“ss”, Landolt et al. 2010), according to Landolt et al. (2010) and Ellenberg and
Leuschner (2010). Hence its presence, associated to algal uncontrolled growth, it is to consider an indicator of slow running waters particularly rich in nutrients/pollutants (eutrophic waters). *Equisetum telmateia* abundance in the riverbed and riverbanks of spring-well C could be instead due to minor nutrient requirements comparing to *Callitriche obtusangula* and to the fact that this species is not salt tolerant (Ellenberg and Leuschner (2010); Landolt et al. 2010). This only partially explains the results of the research, since spring-well C waters have low nitrate, orthophosphate and organic substance content, but a high content in nitrite (0.29 ppm). So, tailor made studies would be necessary to understand if the presence of nitrite in the water (and/or in the soil) can be or not a factor able to favour *Equisetum telmateia* or if this species is instead favoured by the low concentration of nitrate, phosphate and organic substance.

**Management proposals**

Considering this research results, some management suggestions to increase the naturalistic and cultural-historical value of the analysed spring-wells or others in similar conditions are provided below.

The relevant presence of exotic/ruderal species inhabiting land to the disadvantage of native and typical species of Po Valley wetlands is one of the main problems to solve. Fig. 6 shows an operational scheme to remove ornamental/exotic trees and shrubs replacing them with native woody species typical of hygrophilous woods of Po Valley. Removing ornamental/exotic shrubs and trees (excepting the oldest *Morus alba* with an historical-cultural value) must be carried out gradually over time (van Wilgen et al. 2000), starting from shrubs (Fig. 6). Removing the highest ornamental/exotic trees or the ones with the most developed foliage could in fact create clearings favourable to the development of new exotic naturalized plants as *Robinia pseudoacacia* and *Ailanthus altissima* that are heliophilous invasive species (Landolt et al. 2010) found in the spring-wells and in general in a great part of Po Valley (Del Favero 2002; Pignatti 2017; Fogliata et al. 2021). Thus, removing exotic/ornamental shrubs and trees, letting the highest trees, would reduce exotic invasive species growth and allow the implantation of native plants less heliophilous as *Cornus sanguinea*, *Acer campestre*, *Sambucus nigra*, *Corylus avellana*, *Alnus glutinosa*, *Salix alba*, *Populus nigra* and *Carpinus betulus*. These essences should be planted before the vegetative restart (fall, and/or late winter) considering their needs concerning the soil humidity. In fact, some cited trees/shrubs (*Alnus incana*, *Salix alba* and *Populus nigra*) are particular of alluvial forests and need a waterlogged soil (always or periodically) while others are in optimal growth conditions in soils not soaked with water. For this reason, planting the most hygrophilous species in the lower part or spring-wells riverbank (little over the water level) and the others in the higher part (1-2 m over the water level) would be advisable. Besides the cited species (present at least in one of the analysed spring-wells), planting the common oak (*Quercus robur*) would be worthwhile. This oak species is in fact increasingly rare in Po Valley even if it is, together with hornbeam (*Carpinus betulus*), one of the tree species representing the “current potential vegetation” (Biondi 2011) of Po Valley (Del Favero 2002; Verde et al. 2010). Once the young native trees/shrubs have taken seeds and after they created a dense shrubland, it will be possible to eliminate the highest exotic trees and the ones dangerous for people and goods (Fig. 6). Passing the years, the young native trees and shrubs will go on growing and expanding, thus
improving the floristic and ecological features of the spring-wells vegetation. At this stage the management operations of trees/shrubs would be limited to the removal of dead/unstable plants (Fig. 6) and to the vegetation destruction after landslides on the riverbanks (often very steep, Table S1) in extraordinary circumstances. The intervention with low-impact soil stabilization works as soil bioengineering based on plants (or parts thereof) as building materials in combination with dead materials (such as stones, steel, iron, timber, etc.) would be advisable (Bischetti et al. 2014).

While the control of exotic/ornamental trees/shrubs on the riverbanks represents an operation relatively easy, the removal of ruderal and exotic herbaceous species could be much more difficult and expensive (van Wilgen et al. 2000). A great part of the exotic weeds found in the spring-wells are heliophilous species (as for example: *Phytolacca americana*, *Erigeron canadensis* and *Ambrosia artemisiifolia*) recurrent in Po Valley (Celesti-Grapow 2010; Pignatti 2017). Therefore, once built a dense shrubland (see above) and limiting the quantity of light in the herbaceous layer of the vegetation, the most heliophilous exotic and annual ruderals (therophytes) should reduce and the least heliophilous could be removed by qualified technicians (van Wilgen et al. 2000). Sowing or planting young plants of herbaceous species could be planned to encourage the establishment of wetlands. In this case a more structured environmental restoration project involving specialized centres/university could be envisioned. Sowing the native wetlands species of Po Valley could be then an option. However, finding this material in Italy is regrettably difficult (if not impossible), as Italy, comparing to other European countries, has very few producers of native seeds. It is sufficient to consider that in Italy there are only three producers (“Seme Nostrum”, “Centro Flora Autoctona” and “Flora Conservation”) (Giupponi and Leoni 2020) while in Germany there are 12 (Prasse et al. 2010) as well as they are more numerous in Switzerland (SKEW 2009). The shortage of native seeds producers in Italy is due principally to the lack of regulatory instruments to govern the use of autochthonous seeds in restoration work (De Vitis et al. 2017), issue to sort out as soon as possible to favour and improve the restoration of wetlands vegetation (Giupponi et al 2017b, 2019).

For what concerns the riverbeds of the spring-wells (zone I), given the absence of exotic species, possible interventions of re-introduction/re-colonization are the only actions to consider. In this zone, changes of the physical-chemical characteristics of the water and removing/upset the mud layer of the riverbed where hydrophytes as *Callitriche obtusangula* can root are to avoid. While the mechanic disturbance of the spring-well riverbeds is an action relatively easy to control as it is mainly attributable to human cleaning intervention, controlling the chemical-physical water and mud features is more difficult as they depend on both climatic and land use factors (Kløve et al. 2014; Balestrini et al. 2021). In the case study the high nitrogen content (in particular ammonium and nitrite), phosphates and organic substance is reasonably ascribable to the use of frequently employed intensive agriculture products (as ammonium nitrate) or possible industrial/urban discharges. Considering the difficulties of depurating groundwaters (Balestrini et al. 2008, 2018), this issue could be at least reduced identifying and stopping possible uncontrolled spills of discharge water in the spring-wells or nearby areas, and/or limiting the use of polluting chemicals in the fields adjacent the study areas. This last action could be facilitated by instituting one or more protected areas (as local park of supra-municipal interest – PLIS) able to protect
spring-wells creating also buffer areas where only low-input agricultural techniques (as organic agriculture) can be employed. If land managers want to start the depuration and/or protect spring-well groundwaters against pollution in accordance with the European directive (EC 2006), the trade-off showed in this research will be to take into consideration: polluted/eutrophic waters favour the growth (and conservation) of *Callitriche obtusangula* while oligotrophic waters limit this rare species. In this case, a situation of compromise would seem ideal, meaning waters moderately eutrophic (with nutrients/pollutants values possibly under the legal threshold) that however allow the growth of *Callitriche obtusangula*. Finally, for what concerns the water temperature at the spring, the higher values comparing to other research works (Cavagnis and Orsini 1992), could be due to global warming (Kløve et al., 2014), aspect that is extremely difficult to manage in particular at a local scale.

**Conclusion**

This research work characterizes the vegetation of some spring-wells in one of the most industrialized and inhabited areas of Europe, Po Valley. The four studied spring-wells shows similar ecological and floristic-vegetational features, some of which are clear indication of environmental disturbance/degradation while others add value to these environments. The high presence of exotic and/or annual ruderal species as well as eutrophic waters notably containing ammonium (even over the legal limit) and, in some cases, orthophosphate and organic substance are among the disturbance indicators. However, spring-wells shows better floristic and ecological features comparing to the corn fields dominating Po Valley and includes species of historical-cultural value as *Morus alba* and rare, in Lombardy and in general in Italy, as *Callitriche obtusangula*, an hydrophyte requiring slow eutrophic waters. The research results also allowed the formulation of management proposals useful for land managers to enhance the environmental, historical-cultural, and scenic value of the studied spring-wells and other with similar characteristics in Po Valley, deserving valorisation and protection.

**Declarations**

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Authors’ contributions

Conceptualization: Luca Giupponi, Gian Battista Bischetti; Methodology: Luca Giupponi, Gigliola Borgonovo; Formal analysis and investigation: Luca Giupponi, Gigliola Borgonovo, Valeria Leoni, Marco Zuccolo; Writing - original draft preparation: Luca Giupponi, Valeria Leoni, Gigliola Borgonovo; Writing - review and editing: Luca Giupponi, Valeria Leoni; Funding acquisition: Gian Battista Bischetti; Supervision: Luca Giupponi, Gian Battista Bischetti

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Figures

Figure 1

The study area where the four spring-wells are located (a) and the three zones where the phytosociological relevés were carried out (b): I, bed of the spring-well; II, banks and edges; III, maize fields close to the spring-well. The code of the spring-wells (A, B, C and D) is the same reported in Table 1

Figure 2

Biological spectrum of spring-wells flora (a), percentage of exotic and native species (b) and abundance definition of the species found in the spring-wells referring to Lombardy region according to Martini et al. (2012) (c). The photographs show Callitriche obtusangula (rare native species) (d) and Oxalis articulata (uncommon exotic species) (e). Key: G, geophytes; T, therophytes; H, hemicryptophytes; P, phanerophytes; NP, nano-phanerophytes; I, hydrophytes; RR, very rare in Lombardy; R, rare; U, uncommon; C, common; CC, very common
Figure 3

Dendrogram of the relevés (capital letters indicate the code of the spring-well) (a), distribution of total within-clusters sum of squares by number of relevé groups distinguished via hierarchical clustering (b), DCA biplot (c) and variance explained by the first four DCA axes (d). Key: I, relevés performed in the spring-wells bed; II, relevés of the banks/edges vegetation; III, relevés of the maize fields.
Figure 4

Number of species (a) and values of index of exotic component (IE) (b), index of maturity (IM) (c) and ecological index of maturity (EIM) (d) of the three types of vegetation: I, vegetation of the spring-wells bed; II, vegetation of the banks/edges; III, maize fields
Figure 5

CCA ordination biplots of spring-wells (A, B, C and D) associated with water features and species identified in the spring-wells beds. Key: Temp., water temperature; Kubel, organic substance; pH, water pH; NO2-, nitrite; NO3-, nitrate; NH4+, ammonium; Cl-, chloride; PO43-, orthophosphate; Equisetum_t, Equisetum telmateia; Helosciadium_n, Helosciadium nodiflorum; Callitriche_o, Callitriche obtusangula; Glechoma_e, Glechoma hederacea; Solanum_n, Solanum nigrum; Agrostis_s, Agrostis stolonifera; Veronica_a, Veronica anagallis-aquatica; Poa_p, Poa palustris; Lythrum_s, Lythrum salicaria; Phalaroides_a, Phalaroides arundinacea; Lemna_m, Lemna minor; Hypericum_t, Hypericum tetramerum; Algae, algae
Figure 6

Scheme of the work to carry out over ten years to improve the ecological characteristics of the vegetation (trees and shrubs) of the spring-wells banks/edges

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