Wide spread pools and drinking troughs microbiological biodiversity inside the Presidential Estate of Castelporziano (Rome).

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Abstract

Rosselli Team has had the opportunity to study the Presidential Estate of Castelporziano. The Estate of Castelporziano is part of the allocation of the President of the Italian Republic and is subjected to protection on account of its recognized natural and environmental value. Our zero or null hypothesis is that, due to its protection state, the Estate ecological system and its biodiversity is constant in terms of microbiological species in all the water systems. The team decided to collect samples from both natural pools and drinking trough, from wide spread different sites along all the Estate extension. Samples are analysed by microbiological culture approach. Moreover, phisico-chemical analyses are carried out to point out nitrate and organic compounds content as supporting information at microbiological data.

Introduction

The Presidential Estate of Castelporziano lies on the western outskirts of the Capital on the dune-system environment between Ostia and Pratica di Mare, about 20 Km far from the centre of Rome. It has well-marked borders and along most of its perimeter there is an endosing wall. The Castelporziano Estate - Capocotta was annexed in 1985 - preserves the primitive features of the Latium natural coast environment, despite the increasing expansion of Rome towards the sea.

Biodiversity is a fundamental main stone for the survival and development of humanity. Unfortunately, environmental degradation and pollution put at risk the conservation of numerous animal and plant species.

The qualifying element of Castelporziano is represented by its high natural aesthetic and archaeological value. The overall size of the Estate is of 5979.3 ha, mostly covered by forests and Mediterranean scrub. The remaining area is covered by pastures, agricultural area and open natural areas while residential and service areas are only about 173 ha wide. The list of species of animal and plant organisms and microorganisms in Castelporaziano Estate proves the exceptionally high grade of biodiversity in an area which is not extremely large. The list includes 5039 species, divided as follows: 8 Monera, 118 Protists, 722 Mushroom, 229 Lichens; 1044 Plants, 2918 Animals (2380 insects and 538 superior animals). The human impact is minimised within the Estate.

The high degree of biodiversity that characterizes the Estate mainly depends on the presence of wetlands, consisting of 169 pools, of which 88% are of natural origin. These are dynamic areas, sensitive to the influence of natural and anthropic factors, which play a primary role from an ecological point of view as they regulate the balance for the survival of many animal and plant species, of insects, and of the birds that use them as shelter, rest areas, for wintering or nesting. Depending on the size of the water surface and the depth, a zoning of the vegetation, or the succession of strips can be noticed.

The Estate is in a plain area, marked in the coastal area by low dune formations (recent dunes), mixed with depressions (blades), while the remaining area (ancient dunes) is quite uneven; the internal part, with 85 m. high relief in location "Contumaci", is made of alluvial and volcanic soils. The wetlands of Castelporziano develop mainly on land of the ancient

Dune characterized by strong variations in the water level which cause flooding in the winter-spring and complete period drainage in summer. The ancient Dune pools consist of natural depressions in which the collection and permanence of water are favored by low permeability localized layers.
For a bacteriological sanitary point of view, stagnant and current continental water is quite well known both in regard to their content in pathogenic bacteria and to the elimination of human waste, such as sewage water.
About indigenous bacteria, on the other hand, there is only general knowledge. The main difficulties in their study are:
• cultivation, the common culture media used in bacteriology are not normally employable, due to the different nutritional and environmental needs of aquatic bacteria;
• classification, which for these bacteria has not yet been systematically addressed.
The function of bacteria in aquatic environments is very important for the matter cycle. They transform the organic substances of plant and animal offal into inorganic compounds available to plants (mineralization) and they are also a food source for other small organisms, such as protozoa, rotifers, cladocerae.
Bacteria can live in the most extreme environmental conditions such as temperature (from 0 ° C to 80 ° C), pH (from 1 to 13.0) and salt concentrations (range from quite 0 - distilled water - to saturated saline solutions). The concentration of water dissolved oxygen is also important for bacterial processes because obligatory aerobes, facultative anaerobes and obligate anaerobes development depends on oxygen availability in space as in time.

Zero Hypothesis

The ecological system of Castelporziano Estate and its biodiversity is under strictly protection. For this reason, the biodiversity also in terms of microbiological species in all the water systems should be the same. Al least small difference can be pointed out in pools and troughs comparison.
To verify the hypothesis, water samples are collected from both natural pools and drinking trough, from different sites and along a North-South line in order to get samples wide spread along all the Estate extension (see map on PowerPoint presentation). Samples are analysed by microbiological culture approach. Moreover, phisico-chemical analyses are carried out to point out nitrate and organic compounds content as supporting information at microbiological data.

Methods

All the analyses were carried out in the chemical and microbiological labs of Rosselli Institute.
The water samples were collected by sterile glass bottles for microbiological analysis and by plastic bottles for chemical analysis. The glass bottles were previously sterilised by autoclave.

Microbiological Analysis:

Microbiological culture is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions, and are used to determine the type of organism, its abundance in the sample being tested.
An important data concerning bacteria in water is their number for a given volume of water. To establish this, dish counting methods is used (total bacteria count or TBC). It is carried

out by seeding a known water volume on a Petri dish containing a specific nutrient medium. Unfortunately, there is no medium capable of allowing the growth of all bacterial species; the Plate Count Agar (PCA - Tryptic Glucose Yeast Agar) is used in this study. Microbiological cultures were carried out in different conditions. The medium used is PCA for all organisms being unspecific medium and Sabouraud Dextrose Agar (SDA) for fungal organisms. The incubation temperature is 22°C using both media and 37°C in PCA medium in both aerobic and anaerobic conditions. 100uL water solution is seeded into medium Petri dish for each sample collected from the different sites.

Chemical Analysis:

Organic substances by ***Kubel method***: The method consists of indirect titration. An excess of potassium permanganate is added to the water being analyzed and this salt becomes an oxidizing agent for the organic substances present in the sample. By means of subsequent retro-titration, the potassium permanganate which has not reacted is determined and then consequently (indirectly) the concentration of the organic substances present, (expressed as mg/l of oxygen needed for oxidation) is also determined. The oxidizability of Kubel is a nonspecific parameter. ***Substances***: water samples, potassium permanganate (KMNO4), distilled water, sulfuric acid (H_2SO_4), sodium oxalate ($Na_2C_2O_4$). ***Materials***: burets, grippers to spider, flask, graduated cylinder from 5 ml, funnels, pipettes, flasks of 100 and 250 ml. ***Analytical protocol:*** Water which does not contain organic material (usually bi-distilled water, the second distillation made in the presence of potassium permanganate) must be used for the preparation of reagents and for rinsing. Pure sulfuric acid diluted 1:3, potassium permanganate 0,01 N and pure oxalic acid solution 0,01 N are used for the analysis. 5 ml of sulfuric acid and 10 ml (or more) of permanganate must be added to 100 ml of water sample. After 7 minutes of boiling a violet color will appear, indicating the excess permanganate. A volume of oxalic acid equal to the amount of the permanganate is added. Part of the acid in excess will remain in the solution (an amount which is analogous to the permanganate used by organic substances). The liquid fades. The oxalic acid in excess is then hot titrated by a potassium permanganate solution until a persistent pink colour occurs. According to the volume of permanganate used, the mg/l of O2, typical to the present organic substances, are known. In fact 1 ml of 0,01 N permanganate equals 0,00008g of oxygen. The ml used in the reaction are multiplied by 0,08 and thus obtaining the concentration expressed as Kubel. This analytical protocol is interfered with by water containing manganese substances or non organic reducing agents.

Nitrate by UV spectroscopy: Measurement of UV absorption at 220 nm enables rapid determination of NO3-. However, UV light is also strongly absorbed by organic matter at these wavelengths. A two-wavelength approach is adopted and evaluated for the correction of organic matter contributing absorbance. The measurements are carried out at 220 nm and 275 nm. The relationship between absorbance at 220 nm and 375 nm is considered in the nitrate calibration curve. ***Preparation of standard curve:*** Dry potassium nitrate (KNO_3) in an oven at 105°C for 24 h. Prepare nitrate calibration standards in the range of 0 to 50 mg/L by diluting. Treat NO_3^- standards in same manner as samples. To 50 mL clear sample, add 1 mL HCl solution (1N) and mix thoroughly. The samples are filtered before UV analysis.

The ***pH*** and ***conductivity*** measures were carried out respectively by a pH meter (based on a special glass electrode) and a conductimetry.

Results

The microbiological data are reported in table 1.

Sample	Site name	Site Code	PCA medium aerobic/22°C		SDA medium aerobic/22°C			PCA medium aerobic/37°C		PCA medium anaerobic/37°C	
			UFC	species	UFC	species	Mildew	UFC	species	UFC	species
#1	drinking trough Castello		131	3	7	1		51	2	37	2
#2	drinking trough Pepparello		230	5	12	6		6	2	8	2
#3	drinking trough Dogana		195	7	7	3	2	7	4	5	1
#4	Temporary Pool Pignocchetto	120	50	8	3	2	2	295	3	4	2
#5	drinking trough Tor Paterno		24	9	3	3	1	4	1	0	
#6	Pool Paterno Ruderi	147	42	6	8	6	3	24	4	8	2
#7	Temporary Pool Malafede	21	999	2	999	4	1	999	5	120	1
#8	Temporary Pool Della Luce	20	32	8	9	5	1	1	1	3	1
#9	Temporary Pool Ponte Guidone	22	29	7	0	0		9	4	2	2

The chemical data are reported in table 2.

Sample	Site name	Site Code	pH	Conductivity (uS)	Organic compound (mg/L)	$NO3^-$ (mg/L)
#1	drinking trough Castello		7,8	1776	0,48	23,05
#2	drinking trough Pepparello		8,2	1060	1,36	20,34
#3	drinking trough Dogana		8,2	1006	1,28	14,36
#4	Temporary Pool Pignocchetto	120	7,0	153	12,96	nd
#5	drinking trough Tor Paterno		8,0	562	4,56	nd
#6	Pool Paterno Ruderi	147	7,9	228	15,12	nd
#7	Temporary Pool Malafede	21	7,4	149	37,28	4,57
#8	Temporary Pool Della Luce	20	7,1	116	7,04	nd

#9	Temporary Pool Ponte Guidone	22	7,1	106	18,56	nd

nd: not detectable

Discussion

Results point out four types of bacterial populations that can be distinguished in aquatic environment. In small dimension and high content organic substance environment the species grow massively at the surface of the water, due to conditions of surface tension, light, oxygen and temperature. A greater degree of trophy corresponds to a more active bacterial life. Dystrophic pools compared to harmonic pools, whatever their degree of trophy is, are characterized more by differences in bacterial densities than by typical bacterial species. Up to 50% of the cultivable bacteria present in clear pool waters are pigmented, mostly in yellow, but also in purple pink, brown, etc. Pigmented bacteria are instead practically absent in the turbid or dark waters of dystrophic pools.

Results point out a considerable variability between the various samples both from drinking troughs and from swimming pools. Due to the different water source between pools (mainly rainwater) and drinking troughs (groundwater) the data are discussed separately.

The water troughs #1, #2 and #3 are adduced by a pipeline from the same fount at Castello site. The bacterial species are qualitatively and quantitatively similar in the three samples, in particular in terms of the mesophilic anaerobic bacteria and fungi. Greater variability in species is observed in the psychrophilic and mesophilic aerobic charge (see table 1). The sample #5 which is fed by a different fount at Tor Paterno, shows a great difference both with #1, #2 and #3 samples, and inside the sample itself (see table 1) (See photos in PowerPoint presentation).

Moreover, there are differences between the first group of troughs (#1, #2 and #3) and the #5 in terms of phisico-chemical data. The conductivity and nitrate content is higher in the first group then in the other, while the organic compound content points out an opposite trend. These last data are not in agreement with a more active bacterial life related to a greater degree of trophy.

The pH values are quite basic in all the samples. The higher values of nitrates pointed out in Castello drinking trough sample and in the other ones served by the same pipeline, could be due to the human presence in residential and service area such as to the presence of pastures and plantations in close areas.

On the alluvial soils of the northern part of the Estate the groundwater (#7, #8 and #9) is permanent and Phragmiti-Magnocaricetea can be found. Similar but less developed populations, often in association with rushes, can be found in sublittoral wetlands set on the ancient dune (#4). In the coastal area, on the soils of the recent dune (#6), surfacing aquifers in presence of basic sands are present.

From a microbiological point of view, the analysis of the samples (#4, #6, #7, #8 and #9) points out heterogeneous results in all the four microbiological parameters studied. It is interesting to note that the two pools (site code 21 and 120, respectively #7 and #4 samples) with the highest TBC, in particular the mesophilic one, are those located near the roads and in more open spaces and probably those most accessible to mammals that populate the Estate. Moreover, the sample #7 points out the highest value in nitrate and organic compounds content. The nitrate content in #6, #8 and #9 samples is not detectable due to the high organic compound content.

The quite high values of nitrates point out in Malafede Temporary Pool sample, associated to a high species variability, could be due to the presence of pastures. A different pattern is pointed out for the organic compound contents. The higher values are in pool samples in comparison with drinking trough ones. The conductivity measures can be in agreement with the only two main ions studied and measured. The pH values are close the neutrality for pools while they are quite basic for drinking troughs. The pH values and nitrate contents data are in agreement with those published in 2012 (6)

Conclusions

Results point out a considerable variability between the various samples from both drinking troughs and pools supported by a variability in phisico-chemicals data. The zero hypothesis is confirmed only in difference between pools and troughs comparison. Otherwise many differences in terms of microbiological species and physico-chemical parametres point out a disagree with our zero hypothesis.

Results point out that the mesophilic bacterial charge and nitrate content in troughs decrease with moving from Castello (#1) to Dogana (#3). These data confirm the possible effect of anthropic contamination, effects not detected in Tor Paterno trough (#5) which is far from Castello and in a wider area. For what concern pools, the high variability can be related to the specific location referring to close road or path and open areas.

The presence in Castelporziano Estate of a such richness and variability of species is based on a composite mosaic of environments. Such an environmental diversity must be carefully protected and the relationship between man and biosphere must be safeguarded. At the same time, wetlands are also very vulnerable environments. Especially in temperate regions their evolution is strictly connected to the variations of water, from the climatic aspects and from the infrastructural works of man, such as the canalization and the indiscriminate collection of water. Despite their scientific and cultural importance, their conservation and protection are problematic, since they are often too small environments to merit protection and management plans alone.

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