# Microbiological quality of surface waters of Rome and it's County from 1890 to 2010: a systematic review of Roman Hygiene School 

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## ABSTRACT

Research on the quality of surface waters has been performed in Italy during the development of large urban areas, and in Rome this has been the duty of the Istituto di Igiene of the Sapienza University since 1890. Using MedLine - along with traditional consultation of papers printed before 1968 - we identified 100 articles published in the period from 1890-2010. Thirty of them met the inclusion criteria (to have been written by researchers of Roman universities and to contain microbiological information about the surface waters of Rome). The majority of papers identified ( $46.6 \%$ ) were published during the Sixties and Seventies, and $30 \%$ in the twenty years that followed (1980-1999). The most frequent microbiological descriptors were "Total coliforms" and "Streptococci". The body of waters most frequently investigated were the river Tiber and the coastal waters around Fiumicino, where the Tiber flows into the Tyrrhenian sea. The quality of surface waters has always been of central interest to the researchers of the Roman School of Hygiene. The excellent quality of past research, and the renovated interest of International Organizations and of the European Union, should encourage public health researchers to persist in this strategic field of investigation which has strong interconnections with the protection of individual well-being and community health, as well as with environmental preservation.

Key words: Surface water; Water microbiology; Microbial indicators of fecal pollution
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DOI: 10.2427/9471
Published as Online First on February 12, 2014

## INTRODUCTION

Since ancient times, the development of towns settled along river banks was conditioned by the ability to manage their waters, be it the ability to avoid inundation and/or to
control their water supply for many uses such as drinking, sewage disposal, hydropower production and navigation for commercial purposes.

It is well known that the presence of a proper system of sewage disposal and water
supply is the basis for ensuring the health of urban populations. The interest in such issues dates back to the dawn of preventive medicine and, already in the Augustan period, the city of Rome was equipped with an impressive web of aqueducts, a complex system of active removal of used water [1], and a previously unseen provision of public baths and spas.

The progressive decay of this "water culture", so much appreciated by the Romans at the height of the Empire, occurred during the Medieval period, and this contributed to the spread of contagious diseases related to poor personal and community hygiene, which climaxed with typhoid fever and with cholera epidemics. Only in quite recent times(end of XIX century) was the role of water in the transmission of pathogenic agents of epidemic diseases, such as Asiatic cholera [2], clarified.

The urban expansion of Rome after it's annex to Italy resulted in a dramatic population increase [3] and it became necessary to have both the approval of ad hoc environmental and social laws (the Crispi Pagliani Law of 1888) and a detailed Hygiene code, both of which considerably influenced scientific research and consequently led to modern microbial controls of all surface water bodies of the surroundings.

In this context, a lively and intense research activity was carried out by the Roman School of Hygiene, which has continued up to the present day. Such activity has contributed to the surveillance of the principal water bodies in and around Rome, and of the Latium coastal waters as well. The relative scientific production is reported and discussed in the present paper.

## METHODS

The inclusion criteria used for our bibliographic search were the following: Authors belonging to research groups of Roman universities, and the presence of microbiological data relating to the surface waters of Rome and its surroundings. We excluded papers of a strictly methodological nature (such as, for example, analysis of specific sampling methods, of isolation techniques, or other) and Reviews. In any case, those papers not reporting, or re-publishing, original data were also excluded.

The research was carried out electronically with Medline using the following keywords and meshes: surface water, water microbiology,
microbial indicators. The references mentioned in the selected papers were examined for the possible quotation of further relevant articles. In addition to the electronic research, the indices of the following three Italian journals, starting from the date of first publication, were also investigated:
(1) Annals of the Institute of Experimental Hygiene, founded by Prof. A. Celli in 1889, and quoted by Medline under the title of New Annals of Hygiene and Microbiology (New Ann Ig Microbiol) during the years from 1953 to 1988, and under the name of Annals of Hygiene, Preventive and Community Medicine (Ann Ig) from 1989 onwards;
(2) Hygiene and Public Health, founded in 1945 and indexed by Medline as Ig Sanita Publ starting in 2003;
(3) Modern Hygiene, founded in 1908 and indexed by Medline as Ig Mod for the period 1965 to 1970.
Finally, the collections of all papers published by Professors and Researchers of the Institute of Hygiene of the Sapienza University of Rome were investigated, with the objective of identifying all papers published for the Proceedings of National and International Congresses and Conventions, and also published from 1890 to 2010 on Journals of more limited diffusion.

## Overview of the scientific production

A total of 100 papers, published from 1890 to 2010, were found. By searching electronically on Medline, 63 papers were identified; whilst the manual search produced 24 papers which were published in 15 journals in the years 1940-1987. 13 publications arose from the Proceedings of important conferences organized from 1963 to 1986. All these papers were screened using the PRISMA method [4] (Figure 1).

In the first phase, 36 papers were eliminated: one of these because of duplication, one because it was a Review and 34 papers because they were mostly, or exclusively, of a methodological nature. In total, 64 applicative papers, were selected. After careful reading, a further 34 papers were removed: 13 of them because they only concerned chemical parameters; 11 because they were conducted on surface water sampling outside of the Rome area; 3 because full text was unavailable

(published on Congress proceedings never found, or on unavailable journals); and 7 because they did not concern the study of surface waters. Therefore, only 30 scientific papers, published from 1890 to 2004 (Table 1), were left. In Table 1, these papers are listed by year of publication.

The Temporal trend of scientific production (Figure 2) shows an increase in the frequency of publications since 1950, reaching a peak in the decades 1970-1979 and 1980-1989. Only three studies were published between 1990 and 1999. $66.6 \%$ of the papers, in fact, belong to the period between the beginning of the 60's and the late 80 's. In this period, the greatest research efforts were produced, in terms of temporal extension of study periods (with papers covering an average 4 year period each),
of spatial extension with regard to sampled stations throughout the territory ( 276 sampling stations out of a total of 402) and in terms of the number of microbial species investigated. The most frequently used microorganisms to describe microbiological quality of the water were "Total coliforms" (used in 15 of the 30 papers), "Enterococci" (present in 10 of the 30 papers) and "Salmonella spp" (considered in 6 of the 30 papers).

Note on microbial indicators used in the papers
The first investigations on the quality of the surface waters of Rome date back to 1888. In this year, Celli and Scala, thanks to an indirect method based on the evaluation of nitrites and

ammonia, and on microbial count in gelatin, showed a "modest" pollution level in the Tiber river. In the following years the search for water pollution indicators applied methods based on fluidifying and rolled up cultures (Figure 3, [5]). In the late ' 30 's, research of Salmonella typhi and Salmonella paratyphi A, B, C, dysenteric and meta-dysenteric bacteria (belonging to Shigella spp species) were performed by an indirect method based on the identification of bacteriophages capable of specifically lysing the cultures prepared with the single bacteria to be determined. This method was applied both on broth and on plate culture by velvet swab.

During the '50s, the MPN (Most Probable Number) method was introduced for presumptive colibacteria search which would then be confirmed on a Levine culture medium. The studies were always oriented towards searching total or fecal coliforms and towards MCT valuation (Mesofilic Total Concentration). During the ' 70 s , and in particular after 1973, when our Country was hit by a severe cholera epidemic, important papers on Vibrio cholera were published. The first papers adopting standardized methods were presented during the'80s ([6], using 1975 WHO standard methods to search for Yersinia). Few papers were devoted to searching for Enterovirus [7-9].

## RESULTS

The beginnings (1889-1949)
From 1889, when the journal "Annals of the Institute of Experimental Hygiene" was founded by Prof. Angelo Celli at the Royal University of Rome, and until the second world war (WWII), only 4 studies (representing $13.3 \%$ of such scientific production) were retrieved: three studies referred to the Tiber river (two [10, 11] using two sampling stations, and one [12] on three sampling stations] and one focusing on Bracciano and Albano lakes produced by Frisoni, 1900 [13] that respectively used seven and three sampling stations. Among these, the first paper is particularly important, because of the high number of data reported and because results were compared to European research on other river basins.

The first study, commissioned by the hygiene councilor of the Municipality of Rome (Dr. Giulio Bastianelli), was carried out in 1888 by the Director of the Institute of Hygiene, Prof. Angelo Celli, and published in 1890 in the Annals of the Institute of Experimental Hygiene. In this paper, Celli examines all possible sources of water pollution from the source of the Tiber to its mouth at sea. He calculated the sewage (about 60000 kg per day) directly poured into

| PAPERS BY YEAR OF PUBLICATION |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AUTHORS | ```YEAR OF PUBLICATION``` | JOURNAL | YEAR OF SAMPLING | WATER BODY | NUMBER OF SAMPLIMG STATION | $\begin{aligned} & \text { MICROBIOLOGICAL } \\ & \text { INDICATORS(L) } \end{aligned}$ |
| Celli $A$ et al [10] | 1890 | Annlst Ig Sper | 1888 | Fiume Tevere | 2 | 10 |
| Serafini A [11] | 1891 | Annlst Ig Sper | 1891 | Fiume Tevere | 2 | 24 |
| Frisoni P [13] | 1900 | Annlst Ig Sper | 1900 | Laghi di Bracciano + Albano | 7+3 | 16, 27, 21, 28 |
| Acanfora G [12] | 1939 | Ann Ig | non riportato | Fiume Tevere | 3 | 13, 5, 4, 33, 3 |
| Puntoni V <br> et al. [28] | 1956 | La ricerca scientifica | 1954;1955 | Fiume Tevere + Foce Fiumicino | 3+1 |  |
| D'Arca SU [18] | 1957 | Nuovi Ann Ig Microbiol | 1956 | Mare-Ostia Foce Tevere | 4 | 13 |
| Bosco G et al. [19] | 1963 | Nuovi Ann Ig Microbiol | 1960 | Fiume Tevere | 10 | 6 |
| Bosco G [20] | 1963 | Nuovi Ann Ig Microbiol | 1960 | Mare Fiumicino | 8 | 6 |
| $\begin{gathered} \text { Del Vecchio V } \\ \text { et al. [21] } \end{gathered}$ | 1965 | Nuovi Ann Ig Microbiol | 1960-1964 | Fiume Tevere | 10 | 9,13,11, 20 |
| D'Arca SU <br> et al. [29] | 1966 | Nuovi Ann Ig Microbiol | 1960;1962 | Mare - Foce Tevere | 11 | 9,11 |
| Muzzi A et al. [30] | 1968 | Nuovi Ann Ig Microbiol | 1968 | Mare litorale (da Fregene a Castel Porziano) | 8 | 8,13 |
| Del Vecchio V et al. [7] | 1969 | Quaderni Ricerca scientifica | 1969 | Fiume Tevere | 6 | 29, 19, 15, 1, 25, 31 |
| Panà A et al. [8] | 1974 | Nuovi Ann Ig Microbiol | 1970 | Tevere + Foce Fiumicino | $8+2$ | 36 |
| D'Arca SU et al. [31] | 1976 | Ann Sanità Pubbl | 1973 | Fiumi Aniene e Tevere | 1+3 | 8, 13, 12, 32, 35, 30,18, 23, 7 |
| $\begin{aligned} & \text { D'Arca SU } \\ & \text { et al. [32] } \end{aligned}$ | 1976 | Med Mal Infect | 1973 | Fiumi Aniene + Tevere | 1+3 | 9, 13, 18, 7, 32, 35, |
| D'Arca Simonetti A et al. [33] | 1976 | Acqua e Aria | 1974-1975 | Fiumi Tevere <br> + Aniene | 4+1 | 9,13, 18, 7, 32, 35, |
| Melchiorri G et al. [22] | 1976 | Nuovi Ann Ig Microbiol | 1974 | Lago di Bracciano | 1 | 8, 14, 18, 20, 11 |
| D'Arca Simonetti A et al. [23] | 1977 | Nuovi Ann Ig Microbiol | 1974-1976 | marane | 33 | 9, 13, 18 |
| $\begin{gathered} \text { D'Arca Simonetti } \\ \text { A }[24] \\ \hline \end{gathered}$ | 1978 | Nuovi Ann Ig Microbiol | 1974-1976 | marane | 33 | 35, 32, 30, 36 |
| D'Arca Simonetti A et al. [34] | 1979 | Nuovi Ann Ig Microbiol | 1976-1978 | Fiume Tevere + Foce | $5+8$ | 9,14,18, 2, 26 |

the river by the sewerage system subdivided into fifteen separate basins. The sampling points were located upstream (Molle Bridge, now Milvio Bridge) and downstream (Magliana quarter) of the town. Prof Celli concludes by saying that the Tiber, "with rather low waters, before crossing the town, has a modest bacterial concentration, which develops in the urban tract, reaching a peak between St. Paul quarter and Magliana quarter and, therefore, far outside the town, to be reduced before reaching the mouth at Fiumicino to a level comparable to the one measured at Molle Bridge".

His studies explain both the microbial pollution growth along the urban tract of the
river and the self-purification of river waters as they flow from Rome to Fiumicino. Such a process was able to take place at that time thanks to the scarce human colonization of the west Roman countryside, an area extending from the west of the town borders up to the sea.

The historical period which saw the start of these investigations was around 1970, when Rome became the capital of the Italian Kingdom. The city, since 1871, has had to face problems concerning a real demographic boom, largely sustained by migratory flows originating from different Italian districts: initially from two Northern regions - Piedmont and Tuscany, to which

Epidemiology Biostatistics and Public Health - 2014, Volume 11, Number 1

## TABLE 1 (CONTINUE)

| PAPERS BY YEAR OF PUBLICATION |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AUTHORS | YEAR OF PUBLICATION | JOURNAL | YEAR OF SAMPLING | WATER BODY | NUMBER OF SAMPLIMG STATION | MICROBIOLOGICAL INDICATORS(L) |
| Sebastiani Annicchiarico Let al. [35] | 1982 | Nuovi Ann Ig Microbiol | 1978-1979 | Mare Ostia | 6 | 9, 2, 13, 12, 11, 26, 34 |
| Sebastiani Annichiarico L[36] | 1984 | Nuovi Ann Ig Microbiol | 1982-1983 | Fiume Tevere + mare Fiumicino | $7+12$ | 9, 13, 12, 20, 11, 37 |
| Sebastiani Annicchiarico L et al. [6] | 1984 | Nuovi Ann Ig Microbiol | 1982-1983(67) | Fiumi Tevere + Aniene + mare Fiumicino | 4+1+43 | 9,13, 12, 20, 11, 32 |
| Sebastiani Annichiarico L et al. [37] | 1984 | Nuovi Ann Ig Microbiol | 1982-1983 | Fiumi Tevere + Aniene + mare Fiumicino | $24+1+12$ | 12, 20, 11, 32, 38 |
| Mastroeni I et al. [38] | 1986 | Nuovi Ann Ig Microbiol | 1984 | Fiume Tevere | 5 | 9, 13, 12, 20, 11, 32 |
| Sebastiani Annicchiarico L et al. [39] | 1989 | Ann Ig | ND | Fiume Tevere + mare foce Tevere | 2+3 | 13, 12, 20 |
| Divizia M et al. [25] | 1991 | lg Mod | 1989 | Fiume Tevere | 8 | 13, 12, 20 |
| De Vito E et al. [40] | 1994 | Ig moderna | 1991-1992 | Mare Fiumicino | 1 | 13, 12, 20, 11, 32, 36 |
| Donia D et al. [9] | 1996 | Ig Sanità Pubbl | 1991-1992 | Mare Fiumicino | 1 | 36,6 |
| Bonadonna L et al. [26] | 2004 | Ann Ig | non indicato | Fiume Tevere | 1 | $22,13,9,37,12,20,18,32,$ |

1 Adenovirus, 2 Batteri autotrofi, 3 Bacilli dissenterici, 4 Bacillo paratifoide, 5 Bacillo tifoide, 6 Enterofago, 7 Candida e Candidometria, 8 Carica batterica totale, 9 Eterotrofi, Mesofili, CMT, 10 Carica microbica in gelatina, 11 Batteri solfitoriduttori, 12 Colimetria fecale, 13 Colimetria, 14 Eterotrofi 15 Coxakie, 16 Batteri cromogeni, 17 Cryptosporidium spp, 18 E. coli, 19 ECHO, 20 Enterococchi, 21 Batteri fluidificanti, 22 Giardia, 23 Miceti, 24 Microrganismi, 25 Mixovirus, 26 Batteri nitrosanti, 27 Batteri non cromogeni, 28 Batteri non fluidificanti, 29 Poliovirus, 30 Pseudomonas aeruginosa, 31 Reovirus, 32 Salmonell spp, 33 Shigella spp, 34 Tiobacilli, 35 Vibrio colerae, 36 Enterovirus, 37 Aeromonas hydrophila, 38 Yersinia enterocolitica

FIGURE 3
COLTURE ARROTOLATE


From: De Giaxa V. Manuale d'igiene pubblica. Milano: Ed Vallardi, 1889 [5]
the previous capitals, Turin and Florence belonged - later on from Central Italy and, lastly, from the South. A strong demand for new housing for the new middle-class of civil servants coming from Turin and Florence fuelled the building industry, which, in turn, attracted workers from all around. Rome, with 213633 residents registered in the census of 1871 , rocketed to 275637 in the second census of 1881 , and twenty years later, in 1901, to 425000 inhabitants.

The Authorities, on several occasions, attempted to approve a town planning but, despite a building-code approval in 1873 (modified in 1909), building growth continued irregularly and chaotically, especially boosted by speculative factors [14, 3, 15].

In the period between 1901 and 1938 our search did not find any relevant papers, despite the fact that this period, of almost forty years, was characterized by huge development and a significant demographic growth. In fact, Rome in 1920 reached 660000 residents. During the twenty year fascist period, Rome almost doubled its population, reaching 1008083 inhabitants in the census of 1931 [16].

In this period, considerable urban structure changes were realized. Typical episodes included the demolition of some areas of the ancient city districts and the deportation of their inhabitants to other new districts (eg. Acilia districts).

With regard to building expansion, this was no less important because of its environmental and social implications, and, in fact, led to the growth of the so-called "borgate". These popular urban conglomerations were built in order to house the least affluent classes, but also to try to regularize the situation that had been created in the suburbs and inside the city due to peasant (contadini) immigration attracted by the building site work. Such immigrants built precarious, abusive and unstable buildings, the so called "baracche", with a population estimated to be more than 50000 units [17].

The study conducted by Acanfora [12] in 1939 closes a forty year period of apparent research inactivity. Perhaps the most important achievement of the Acanfora study concerns the search for bacteriophages in the Tiber waters. These kind of micro-organisms, which produce a lytic activity against "meta-dysenteric" bacteria, were used to indirectly demonstrate the presence of those pathogens which were commonly used as indicators of water pollution.

## The period from 1950 to the present

During the period of the Italian economic boom (from the early' 50 s to the end of the ' 60 s ), the improvement of social conditions spurred the proliferation of "spontaneous" residential areas, all in the suburban belt: in 1953 there were already 31 of them. The demographic growth continued in the postwar period and Rome reached, in the censuses of 1951 and 1961, 1651700 and 2188160 inhabitants respectively, more than double the population of 30 years earlier [17]. In this period, the demographic growth was largely sustained by immigration.

Most of the studies on the microbiological quality of the surface waters were conducted by Roman epidemiologists from the beginning of the post WWII period until 2004, and their contribution is equivalent to $83.9 \%$ of the whole scientific production found by the present investigation.

In the first of these studies, D'Arca in 1957 [18] measured $E$ coli pollution with the MPN method in four sampling stations on the Lazio coast. The study highlighted a massive pollution of coastal seawater, concentrated along the three kilometers extending south-east of river Tiber's mouth.

A further study, conducted by Bosco et al in 1963 [19], evaluated phagic activity as a fecal pollution indicator, and found a very high activity at the Tiber's mouth. The same Author, in a later study of the Tiber [20] along ten sampling points, observed the presence of lythic activity of bacteriophages against Shigella flexneri and Salmonella paratyphi B upstream of the city, while, downstream, he observed the presence of bacteriophage activity on Salmonella paratyphi A, Salmonella typhi and Shigella Shiga, whose concentration resulted highest downstream.

Another important study in 1965 [21] showed that the Tiber's waters were polluted with colibacteria (again measured by the MPN method) with a concentration of $1000 / \mathrm{mL}$ upon their arrival at the northern outskirts of Rome. They also reported that an increase took place after the confluence of the Aniene river (up to $15000 / \mathrm{mL}$ ), and that pollution levels almost doubled as the two major collectors poured their untreated sewage into the river ( $25000 / \mathrm{mL}$ ).

In 1969, Del Vecchio presented findings to the Franco-Italian-Monegasque Congress and
the results of his studies on the presence of Enterovirus in the Tiber waters suggested that these microorganisms could be used, in the future, as indicators of fecal pollution [7].

From 1970 to 2000, an extension of the research from the Tiber (and near its mouth areas) to other surface water bodies located on the outskirts of Rome was seen. One of these studies evaluated the microbial contamination of Bracciano lake, whose waters partially contribute to Rome's water supply.

The results of the research carried out by Melchiorri et al in 1976 [22] showed an Enterovirus absence and a moderate fecal pollution of the lakes of the roman area. Afterwards, research on environmental pollution focused on small streams, defined "Marrane", which flow within the (then) partially urbanized areas of the Rome suburbs. Two studies were published in 1977 and 1978 [23, 24], with sampling carried out in 33 stations of various Marrane. The results described a fecal pollution with coliform concentrations of $105 / \mathrm{mL}$ and $E$. coli values of $104 / \mathrm{mL}$.

In 1984 Sebastiani Annicchiarico et al [6] conducted a study of bathing waters off the Roman coast, finding a Salmonella pollution higher in water than in sediments. There was a significant association between Salmonella presence and fecal coliform concentration, confirming the importance of the latter microbes as indicators of fecal pollution.

Divizia et al in 1991 [25], while testing the Tiber waters in 8 sampling stations, found high levels of chemical organic pollution, accompanied by the highest rates of fecal contamination, probably due to the inadequate waste water treatment system of the town.

The last study included in the review was carried out in 2004 [26] in one sampling station on the Tiber. The results of this study showed contamination by Giardia and Cryptosporidium spp, whose concentrations, however, did not correlate significantly with concentrations of traditional fecal pollution indicators. High concentrations of Salmonella, Bacteriophages and Clostridium spp were also evidenced.

## FINAL CONSIDERATIONS

Form our literature review, it can be seen that there was an early and permanent interest shown by the Roman School of Hygiene
members in the environmental conditions of their own territory, and in particular in the health conditions of the Tiber, of its tributary rivers and of the coastal seawaters.

Accordingly, it must not be forgotten that in the occasion of the official opening of the Institute of Experimental Hygiene in 1885, Prof Corrado Tommasi Crudeli, its first director, delivered a series of lectures on the modifications of the climate of Rome, discussing its inter-dependence with the surface waters regime and its effect on the health of the Roman population [27].

On the other hand, the history of the architectural development and the urban design of Rome - as capital of the Kingdom, and later of the Republic of Italy - was characterized by a substantial messy development, despite the formal adoption in 1873 of an admirable city plan [14, 3, 15]. The disorganization got worse after World War II, and left us a legacy of periphery conurbations of very bad quality and without sewage networks, which had to be built in the years that followed.

Obviously, the surface waters of the Tiber and Aniene rivers have suffered heavily. The history of the sewers of Rome, is hardly exemplary: built late, often malfunctioning and also unable to cope with the changes of flow. They still have problems at present, as shown by the recent fine issued by the Court (June 2011) to the sewage treatment plant of North Rome, because it spilled purified sewage mixed with mud into the Tiber.

The contribution of the hygienists of the "Sapienza" and other universities of Rome, both in the past and more recently, is characterized by their attempt to tackle environmental issues with great attention whilst always providing decision makers with the results of modern and updated scientific research that help them to make decisions on how to improve the system.

If in recent years the interest of hygienists has tended to move to other research projects (epidemiology and prevention of chronic diseases, organization and evaluation of health services, molecular epidemiology, etc.) it is not because environmental issues have lost their appeal, but because the theme - from an exclusive pursuit of hygiene - has become a patrimony of other disciplines also. For instance, sanitary engineering has the responsibility to plan the plants to abate pollution. However, the topic is still important for Public Health,
and therefore for Hygiene, because other unresolved problems arise (such as the revision of pollution indicators, and the taking into account of viruses and parasites also) which provide the opportunity for a renewed interest by the discipline.

It is not by chance that, recently, the Italian Society of Hygiene, Preventive Medicine and Public Health has activated a new Working Group dedicated to "Health and Environment",
as a testimony of this renewed interest and the will to drive the involvement of the Society members in that direction.

This paper is an English translation of the one published in Ann Ig 2011; 23 (6): 44556 (La produzione scientifica della Scuola Romana di Igiene sulla qualità microbiologica dei corpi idrici superficiali di Roma e Provincia dal 1980 al 2010. Una review sistematica).

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