The quality of discharges of public wastewater treatment plants and the *Sludge Biotic Index* for activated sludge: integrative assessment

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Abstract
Wastewater treatment plants (WWTPs) are controlled by Environmental Authorities usually with reference to the compliance to discharge limit values fixed by national and local regulations. An integrated approach (Ostoich et al., 2010) is necessary to achieve the objectives established with the Directive 2000/60/EC (WFD) considering the ecological status of the receiving water body and the discharge quality. Moreover integrative information on the behaviour of the activated sludge in the aeration tank can be useful for plant managers as well as for control Authorities. The study presents the experience on WWTPs control considering the discharge analytic controls and the monitoring activity of the *Sludge Biotic Index* (SBI; Madoni, 1994) for activated sludge. Data from monitoring in the period 2008-14 on SBI values, chemical and microbiological data on the discharges of a sample of n. 35 WWTPs in the province of Venezia (North East Italy) are presented and discussed. SBI appears a useful index for an integrative assessment of the plant functionality, in addition to chemical and microbiological analysis, in particular to monitor and identify critical situations that can determine overtaking of discharge limit values. The SBI method, in an integrated control approach, can be used for small and medium size WWTPs treating only domestic wastewaters, allowing in a case by case assessment even to substitute part of the analytical controls in plant monitoring.

Keywords
Activated sludge process (ASP); Sludge Biotic Index (SBI); bacteria; protozoa; wastewater treatment plant (WWTP); discharge limit value.

CONTROL OF WWTPs AND INTEGRATED APPROACH

Human life and activities produce wastewaters that generally are referred as “urban wastewaters”, that is the mixture of metabolic residues from dwellings and drainage waters. Wastewater from domestic settlements is generally treated with activated sludge processes (ASP), that is able to perform the biological transformation of dissolved and colloidal organic compounds into biomass. ASP is formed of bacteria and other organisms naturally present in water bodies that can utilize dissolved and colloidal organic matter and nutrients to grow and produce biomass; when bacteria and other organism are grown the biologic biomass can be separated from remaining water by settling. Residual WW should be discharged in compliance with the specific limit values defined, in Italy, with the *River Basins Management Plan* (RBMP) and the *Water Protection Plan* (WWPs), prepared respectively by the distric/basin water Authority and by the Region according to the Water Framework Directive 2000/60/EC (WFD; EC, 2000).

Biological treatment processes are based on the natural self-purification process, performed by bacteria and microfauna already present in the water body. In the microfauna, protists are of main importance for an effective treatment of the organic load. Foissner (2016) observes that for the use of protists as bioindicators in WW treatment three different periods can be identified: 1) age of discovery and exploitation (discovery and use of activated sludge for wastewater purification); 2) age of bloom (development of saprobic index and practical tools for performance of sewage plants); 3) age of decline. According to this author we are now in the third phase but his conclusions are that study of protists in ASP systems can still give a valuable contribution to WWTPs performance and management. Ostoich et al. (2010) proposed an integrated control approach on public WWTPs through documentary, technical, management and analytic controls and through functionality verification to assess the plant reliability to guarantee the compliance to discharge limit values. In the official controls on WWTPs discharge analytical control is not always enough to obtain a satisfying picture of the real situation and of the reliability of WWTPs. To monitor the performance of ASP plants Madoni (1994) proposed the *Sludge Biotic Index* (SBI), defined as an “objective index” like the *Extended Biotic Index* (EBI, Woodiwiss, 1980) for rivers. SBI is based on the consideration that the analysis of microfauna can give information in a quantitative way and in standardized form on ASP plant performance. The SBI is based on both structure and abundance of the micro-fauna present in the activated sludge in mixed liquor.

This study presents experimental data from institutional control activity in which analytical control on the effluent as well as SBI on activated sludge have been performed. Case studies and usefulness of the index are discussed in particular for plants along the coast in Veneto Region (province of Venezia, North East Italy). A preliminary investigation on a sample of n. 35 small (<2.000 PE), medium (<10.000 PE) and large WWTPs in the province of Venezia in the period 2008-14 has been performed by institutional control Authority (Veneto Regional Environmental Prevention and Protection Agency-ARPAV). The reliability of WWTPs to avoid overtaking of discharge limit values appears very important in particular along the coast where there can be a direct impact on the quality of bathing waters, which are of main interest for touristic purposes. Integrative SBI analyses can be useful to support functionality analysis. In small and medium size WWTPs, SBI investigation can be integrative to normal planned control activities or can be used in substitution of some analytical controls, with the aim to gain more information on the reliability of the plant to guarantee an effective treatment and satisfactory performances.

Activated sludge processes and the sludge biotic index (SBI)

Protozoa play an important role in the trophic web in aerobic wastewater treatment processes (Curds, 1973; 1982). The biomass in the oxidation tank is really a complex ecosystem and its efficiency in organic load removal depends on its structure. In ASP decomposers are nearly the 91% of the microbial population, while consumers are the last 9%. Biotic indices have been developed for the evaluation of water bodies quality according to the health state of their ecosystems (Madoni, 1994). Ciliates can be used as indicators of effluent quality (Curds and Cockburn, 1970; Curds, 1982) and microsopic analysis can be applied to AS to estimate the quality of effluents (Curds and Cockburn, 1970a). Changes in the structure of the community of AS reflect changes in WW quality and the relationship between protozoan species in aeration tank was quantified to predict plant’s performance (Al-Shahwani and Horan, 1991). The majority of ciliates present in biological WWTP feed upon dispersed populations of bacteria (Madoni, 1994). Protozoa analysis is used for the determination of organic load: low organic loading rate is associated with long sludge retention time, poor feeding substrate and with low number of dispersed bacteria, high diversity with predominance of crawling and attached ciliates (Salvadó and Gracia, 1993).

Increase of organic load is able to modify the microfauna structure with the decline of species diversity and the domination of taxa like small flagellates, free swimming ciliates and attached ciliates (Madoni et al., 1993). Plant behaviour and performance can be predicted on the basis of AS and protozoa analysis (Al-Shahwani e Horan, 1991; Madoni et al., 1993; Salvadó et al., 1995; Drzewicki and Kulikowska, 2011). Salvadó et al. (1995) presented the relationship between the ciliate population density and effluent quality in activated sludge plants and analysis of ciliate protozoa and small metazoa. The SBI method is based on the taxonomic identification of Protozoa in activated sludge with optic microscopic analysis, already used to estimate the quality of the effluents and the performance of the plant (Madoni et al., 1993). For this method it was assumed that the dominance of key-groups and the abundance and number of indicator taxa of microfauna in AS vary depending on the physical-chemical and technological parameters and on the effects of the treatment process. Madoni (1994) proposed and applied a table with double entering, horizontal for diversity and vertical for population density. Protistan ciliates in AS are classified into three functional groups: free-swimmers; crawling forms; attached forms. Performance of activated sludge sewage treatment process depends on the density and the structure of the protistan community on the sludge flocs in aeration tank (Madoni, 2000), but no information is given on dysfunction in the secondary settlement tank (like bulking, rising, pin-point, etc.).

The microfauna considered in SBI have a cosmopolitan distribution; it is assumed therefore that the index SBI is applicable to the activated sludge plants all over the world. SBI appears as a standardized method but, as presented by Drzewicki and Kulikowska (2011), the response is not always simple and linear; in fact, not only there are structural, design and operational factors that must be considered and that can influence the plant performance but for some ciliates classification can be not easy (see Madoni, 1996; Madoni, 2000). Toxicity for activated sludge microfauna of different substances has been observed by different authors, for example see Papadimitriou et al. (2007), Madoni et al. (1996), Madoni (2000), Nicolau et al. (2005), Drzewicki and Kulikowska (2011). Particularly toxic for activated sludge are heavy metals (Madoni et al., 1996): Cd, Cu, Pb, Ni, Cr(VI) can affect negatively the aerobic processes already at level fractions of ppm or even of ppb (Madoni and Romeo, 2006). Among the other inhibiting substances that can be found in industrial discharges there are chlorine, cyanides, phenols, formaldehyde, herbicides and phyto-pharmaceuticals. As a general consideration, Foissner (2016) observes that for the SBI, as for the *Saprobic Index*, it appears not possible to reduce the organism communities to a single numerical value; in fact these indices should be interpreted cautiously. The same author suggests that an effort should be made in the integration of biological analysis on activated sludge, operational conditions, physical-chemical data, technology applied and design data.

MATERALS & METHODS

The WWTPs selected for the study

The WWTPs considered in this study are localized in the province of Venezia (North East Italy; **figs 1a** and **1b**). The Veneto Regional Environmental Prevention and Protection Agency (ARPAV) performed in the period 2008-14 the analytical controls on the final discharges (chemical and microbiological measures) and SBI determinations. Information on selected WWTPs have been supplied by the Veneto Region Environmental Informative System (SIRAV) – Cadastre, managed by ARPAV and fed during control activity. In **tab. 1** the WWTPs census in the province of Venezia is reported. In **tab. 2** the n. 35 WWTPs for which SBI was determined in the period 2008-13 (in one case till 2014) are listed with their potentiality (PE). To understand the operative and performance conditions data from plant managers have been requested and used for the following WWTPs: Venezia-Fusina, Venezia-Campalto, Venezia-Lido and Chioggia plants. Therefore a deeper and more complete assessment is possible only for these plants, although with differences between them.

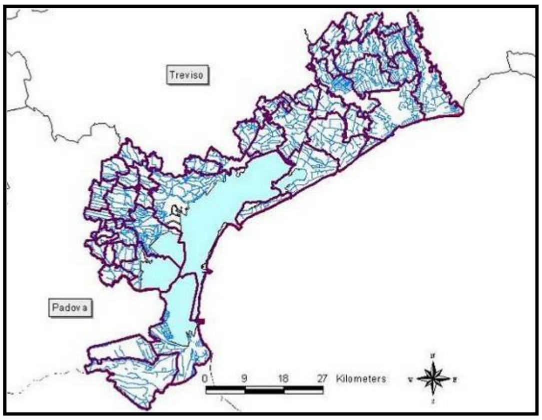

Figure 1a and b. The area of study (province of Venezia) in Veneto Region, North East Italy

Table 1. Number of WWTPs (census) in the province of Venezia distributed in classes of potentiality in PE (Source: SIRAV Cadastre, 2016)

Province	< 2.000 PE	2.000 – 9.999 PE	10.000 – 49.999 PE	>= 50.000 PE	*Tot. Prov.*
Venezia – Numb. of WWTPs	22	18	5	9	54

Table 2. WWTPs investigated for SBI determination - Period 2008-14 – Province of Venezia

WWTP	PE	WWTP	PE
ANNONE VENETO, Capoluogo	2.000	NOVENTA DI PIAVE, Capoluogo, Via Torino	4.500
CAORLE, Capoluogo, Via Traghete	120.000	SAN MICHELE AL TAGLIAMENTO, Capoluogo, via A. Moro	8.000
CAORLE, San Giorgio	3.000	SAN STINO DI LIVENZA - LA SALUTE, via L. da Vinci.	2.500
CAVARZERE, Via Piantazza	17.500	PORTOGRUARO, loc. Destra Reghena, Viale Venezia	8.700
CAVARZERE, Rottanova	1.000	PRAMAGGIORE, Blessaglia	2.500
CAVARZERE, S. Pietro	1.000	GRUARO, La Sega via Giai	2.800
CEGGIA, Via I Maggio	5.000	MUSILE DI PIAVE, Capoluogo via Rovigo	10.000
CINTO CAOMAGGIORE, Via dei Prati	2.000	SAN STINO DI LIVENZA, Capoluogo, via Canaletta	10.000
CHIOGGIA, Val da Rio	160.000	SAN DONA´ DI PIAVE, Capoluogo via Tronco	45.000
CONA, Pegolotte	6.000	TORRE DI MOSTO, via Xola	3.000
CONCORDIA SAGITTARIA, Capoluogo, Via Basse	3.300	VENEZIA, Fusina, via dei Cantieri	400.000
CONCORDIA SAGITTARIA, Capoluogo via Gabriela	3.000	VENEZIA, Campalto, Via Scantamburlo	130.000
ERACLEA, Ponte Crepaldo	4.700	VENEZIA, Lido di Venezia, via Galba	60.000
ERACLEA, loc. Eraclea mare, via Pioppi	32.000	JESOLO, via Aleardi	185.000
FOSSALTA DI PIAVE , Capoluogo via Cadorna	3.600	BIBIONE, via Parenzo	150.000
FOSSALTA DI PORTOGRUARO, Villanova, Via Zecchina	1.800	QUARTO D´ALTINO, Capoluogo via Marconi	50.000
FOSSALTA DI PORTOGRUARO, Capoluogo, Via Europa	3.000	CAVALLINO – TREPORTI, via Fausta	105.000
MEOLO, Capoluogo, via Marteggia	9.000		

Sampling, analytical methods for chemical parameters and Sludge Biotic Index (SBI)

Official sampling and analytical methods adopted in Italy were applied during this study: *Analytical methods* (APAT, 2003) used since 2004. Where analytical methods were lacking in the Italian national legal framework international official methods were also used (i.e. APHA & AWWA 1998). The sampling techniques were the following: instantaneous sampling for microbiological parameters and mean-composite sampling on a 24 hours basis for WWTP effluents. When data are reported as lower than LOQ (<LOQ) the value of the parameter has been substituted with half of its value as suggested in literature to allow elaborations (Spaggiari & Franceschini, 2000).

The SBI method has been set up on the basis of the relationship occurring between plant performance and operating conditions on one hand and the structure of microfauna within the activated sludge reactor on the other. The index is calculated with the use of a double entrance table (Madoni, 1994). The SBI value, from 0 (worst quality) to 10 (best quality), can therefore be determined in the intersection of the selected line with selected column; the index values are grouped into four large quality classes from I (the best) to IV (the worst) (Madoni, 1994; Drzewicki and Kulikowska, 2011). Sludge observations have been performed within five hours from sampling. An optic microscope 100X has been used. First a screening identification has been performed for the present forms in the sludge sample and then the estimation of the relative abundance of each species or group, with a complete count of the forms present in a known volume of sludge.

RESULTS & DISCUSSION

SBI data for the WWTPs' sample

In the period 2008-2013 (2014 for the Venezia-Fusina plant) SBI has been determined for n. 35 WWTPs in the province of Venezia as listed in **tab. 2**. The frequency distribution of SBI scores is reported in **fig. 2a**, while the frequency distribution of the corresponding quality classes are reported in **fig. 2b**. SBI scores are reported in **figs 3** and **4**. The majority of the SBI scores are in range 8-10 an therefore they are in the first quality class (class I), according to Madoni (1994). Despite this good result with reference to activated sludge quality, there are scores lower than 8 down to 3 for n. 15 WWTPs on the whole sample of n. 35 plants, as reported in **tab. 4**; from this table it is possible to see that SBI scores below 8 are found in large as well as small plants. It appears that very small plants are subject to phases of lower efficiency (there is less maintenance and/or control; there can be fluctuations of the entering flow and/or fluctuations of the organic load).

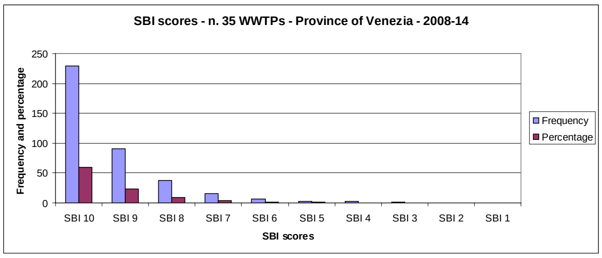

Figure 2a. SBI scores for 35 WWTPs

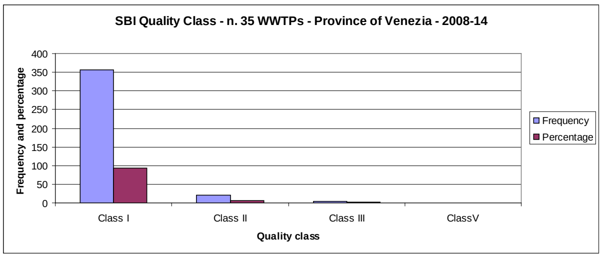

Figure 2b. SBI Quality classes for WWTPs

Chemical quality of the final discharge

For a complete assessment of the causes of the behaviour registered with SBI investigation on the sample of n. 35 WWTPs in the period 2008-15, analytical data on the final discharges have been assessed (only on n. 32 of these plants analytical data on the final discharge were available, according to institutional control activities performed by ARPAV). For reason of space the chemical parameters' trends of only some of the investigated WWTPs with SBI scores have been here presented and specifically: Venezia-Fusina, Venezia-Campalto, Venezia-Lido and Chioggia. The trends of parameters BOD_5, COD and TSS are presented in **figs 5a-b-c-d**. Limit values for discharges have been introduced only for indicative purpose, as each discharge must be assessed according to the vulnerability of the specific area of discharge as established by the regional *WPP* (BOD_5,=25 mg/l; COD=125 mg/l; TSS=35 mg/l).

Table 4. WWTPs with SBI value lower than 8 in the province of Venezia – 2008-14

WWTP	WWTP capacity in PE	N. cases SBI < 8	SBI Scores
Bibione S.M. Tagliamento	150.000	3 cases	7
Venezia - Campalto	130.000	2 cases	7
Caorle – Via Traghete	120.000	1 case	6
Cavallino-Treporti	105.000	2 cases 1 case	6 7
Cavarzere – San Pietro	1.000	1 case	4
Cavarzere - Rottanova	1.000	1 case 1 case 1 case 1 case	7 5 4 3
Chioggia Val da Rio	160.000	2 cases	7
Cona	6.000	1 case	6
Eraclea Ponte Crepaldo	4.700	1 case	7
Fossalta di Piave	3.600	1 case	6
Jesolo	185.000	1 case	7
Venezia-Lido	60.000	1 case	7
Musile di Piave	10.000	1 case	7
S. Stino di L. la Salute	2.500	1 case	6
Torre di Mosto	3.000	2 cases 1 case	7 5

Analysis of the activated sludge microfauna

Among the n. 35 plants on which SBI investigation has been performed by ARPAV, n. 4 plants managed by the same society have been selected for a deeper analysis with plant manager data (self-monitoring); the selected plants are (see **tab. 2**): Venezia-Fusina (400.000 PE), Venezia-Campalto (130.000 PE), Venezia-Lido (60.000 PE) and Chioggia (160.000 PE). The wastewaters treated in these plants are not homogeneous (they are domestic, mixed domestic and industrial); moreover the plants of Lido and Chioggia are subject to strong fluctuations (hydraulic and organic load variations) due to the touristic presences in summer period. The considered parameters supplied by plant manager are: concentration of influent and effluent wastewaters and in particular the mean concentration in the period 2008-14 (values measured in the sampling date for SBI determination) of the parameters BOD_5, COD, TSS, N_{tot}, P_{tot}, N-NH_4, N-NO_2, N-NO_3, N-NO_3, TKN, entering into the plant and treated flows, VSS-volatile suspended solids in the aeration tank, sludge load, sludge age and the concentration of SS in the aeration tank (MLSS).

For many cases it was not possible to obtain the required functional information/data from the plant manager like: the treated flow, entering flow, VSS in aeration tank, TSS, sludge load and age, etc. With reference to the measures performed on the activated sludge, beyond the SBI values, the following parameters have been considered: microfauna density with reference to the protists present in the activated sludge, the total number of systematic units present and the relative dominant groups, the floc's diameter. All these values have been organized for each sampling date of the discharge and of the activated sludge. It must be observed that this study is preliminary and had the aim to see if it was possible to identify a relationship between the sludge status and the functional and management parameters used in the plant operational activity; in a second phase a statistical study will be performed to find out the relationship – if existing – between the SBI and the main functional parameters. Analytical data supplied by plant manager have been produced by the same laboratory for all the four selected WWTPs. The parameters BOD_5, COD, P_{tot}, N_{tot} and TKN have been determined on samples taken in the same date of activated sludge sampling for SBI determination.

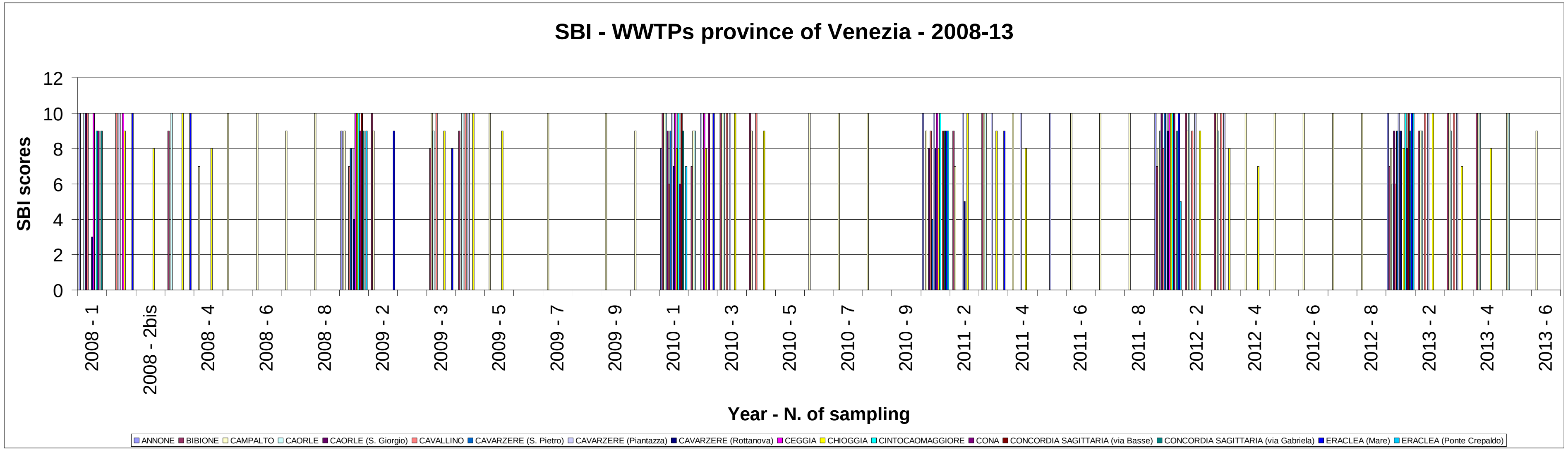

Figure 3. First group of 17 WWTPs in the province of Venezia – SBI scores – 2008-13

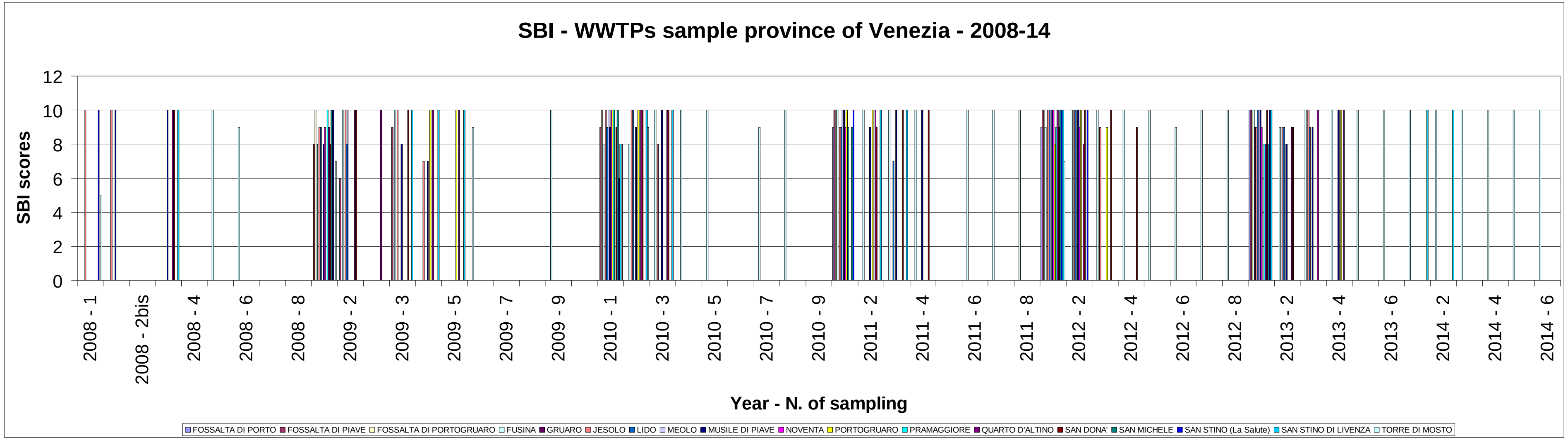

Figure 4. Second group of 18 WWTPs in the province of Venezia – SBI scores – 2008-14 (2014 only for Venezia-Fusina plant)

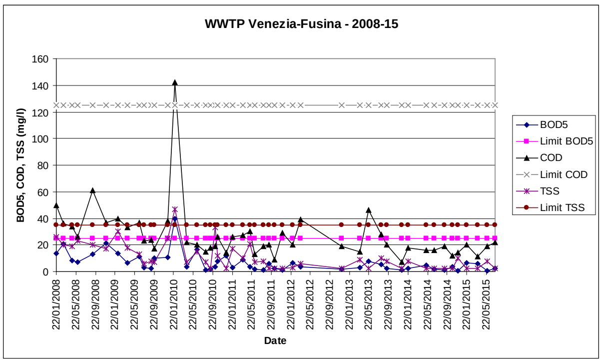

Figure 5a. Trend of WWTP discharge 2008-15

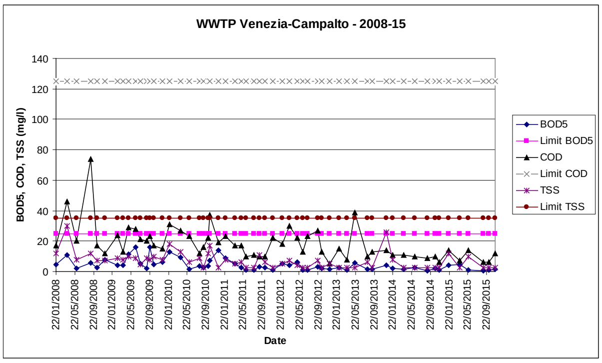

Figure 5b. Trend of WWTP discharge 2008-15

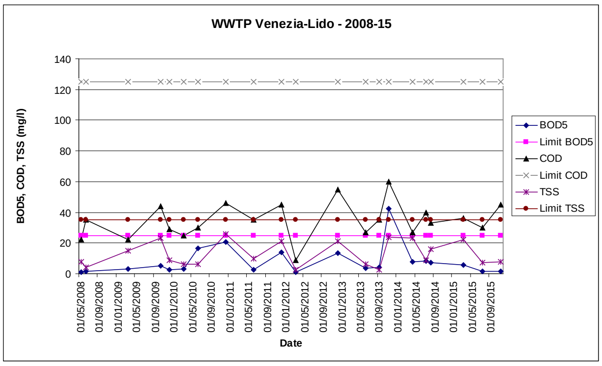

Figure 5c. Trend of WWTP discharge 2008-15

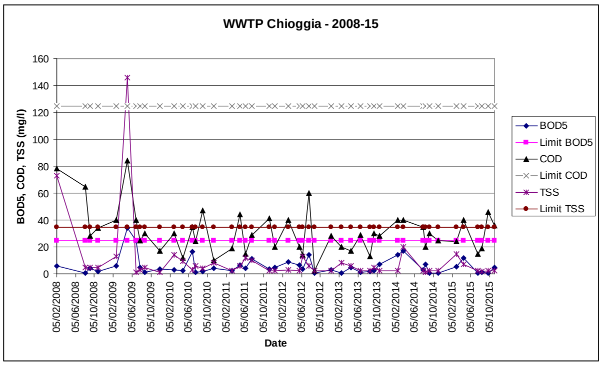

Figure 5d. Trend of WWTP discharge 2008-15

Analysis of data supplied by plant manager

Some derived parameters have been determined like the efficiency of organic load removal (in terms of COD removed), obtained through the comparison of data on influent and on effluent, the efficiency of N removal (as N_{tot}), the efficiency of P removal (as P_{tot}), the ratios COD/BOD_5, C/N, N/P, and, when possible, the SVI (sludge volume index) value. The values of these parameters have been compared: from their trends a relationship can be observed. In **figs 6a, b, c, d** the microfauna characteristics and functionality parameters of the WWTPs, supplied by plant managers are reported for the n. 4 selected plants; only for Venezia-Fusina and Venezia-Lido graphs of microfauna as well functional parameters were possible, while for Chioggia and Campalto they were incomplete and therefore not reported. On the basis of the analysed data derived from the selected four WWTPs, some anomalies are evident and should be investigated more deeply using new physical and process data. As an example the case of the measured parameters in the Venezia-Fusina WWTP on the date of 07/06/2010: in this case unless SBI had a score of 10, removal efficiency had values lower than mean values registered in other period of the year (N removal efficiency have been registered η_{Ntot}=0,73, organic load removal efficiency η_{COD}=0,87, P removal efficiency η_{Ptot}=0,68). Moreover the situation registered on the date 3/12/2013, characterized with a SBI value of 10 too, a number of systematic units of 16 and values of the removal efficiencies respectively of η_{Ntot}=0,63, η_{COD}=0,87, η_{Ptot}=0,74. At the same time, also changing WWTP, interesting situations to be more deeply understood are evident: in the Lido WWTP for example on the date 12/12/2012, with a SBI score of 10 and a number of systematic units of 11, the following removal efficiencies have been registered: η_{Ntot}=0,53, η_{COD}=0,73, η_{Ptot}=0,74.

CONCLUSIONS

The *Sludge Biotic Index* (SBI, Madoni, 1994) has been applied as a useful monitoring and management tool that can assess the health of activated sludge in aerobic wastewater treatment processes. According to Papadimitriou et al. (2007) SBI calculation can be effectively applied for treatment of municipal wastewater, while it is not the case when toxic substances (inorganic or organic) are present. The SBI can be used to monitor and assess the plant performance, organic and suspended solids load reduction, to control operative condition and the reliability in the compliance of discharge limit values in particular of small and medium size plants, treating only domestic wastewaters. Often particular situations can be found with high values of SBI, high diversity of ciliates protozoa present, good characteristics of the activated sludge, but not satisfactory removal efficiencies. These situations could be tied to specific environmental factors: variations of T, pH, presence of toxic substances or growth inhibitory substances, variation of the nutrient composition, etc., or structural and/or management elements.

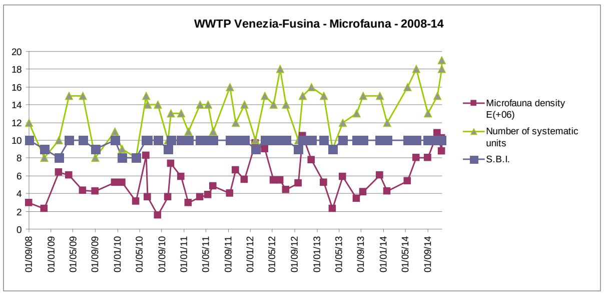

Figure 6a. Fusina – Microfauna characteristics

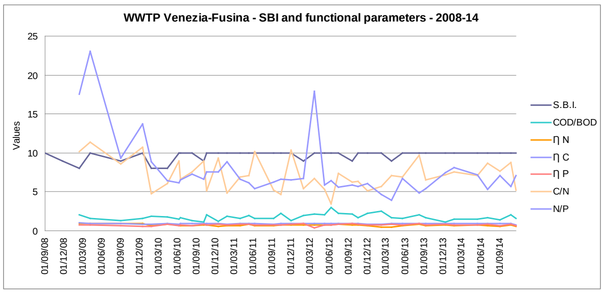

Figure 6b. Fusina – Functional parameters

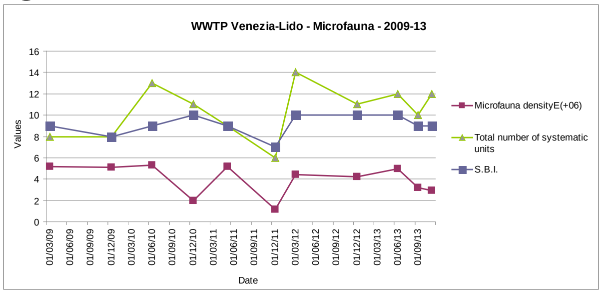

Figure 6c. Lido - Microfauna characteristics

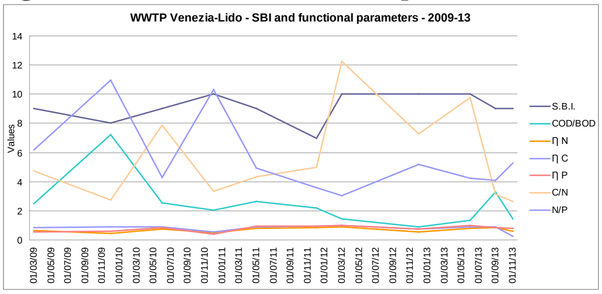

Figure 6d. Lido - Functional parameters

The capability of ciliates as indicators of effluent quality is limited by a number of factors like over or under-loading (Salvadó et al., 1995) or shock organic and ammonium loadings (Drzewicki and Kulikowska, 2011). These aspects make understand that SBI is able to give very useful indication on plant performance but cannot be used alone. It appears useful as an integrative management and control tool. In particular for small and medium size WWTPs it can be used to reduce analytical control frequency once a specific correlation parameter has been determined for each plant and after a significant historical series of data is available for discharge characterization. The general situation with SBI of n. 35 WWTPs in the Province of Venezia (North East Italy) has been presented as a preliminary investigation performed in the period 2008-14; for only four WWTPs of this group functionality data have been supplied by the plant manager and the main findings of the assessment have been the following: 1) the SBI confirmed the satisfying performances of the considered four WWTPs, as evaluated with physical-chemical and microbiological analysis in the same period; 2) the activated sludge analysed for SBI assessment was determined within the quality class I, which corresponds to a very well colonized and stable sludge, with excellent biological activity and consequently very good abatement efficiency (SBI scores ≥ 8), with only few cases of lower classes. This investigation must be considered "preliminary" as more information is needed in collaboration with plants' managers to gain and assess data on abatement efficiency. In particular a statistical analysis on the identified parameters is necessary but it has not been performed in this study because of lacking of data.

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