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RESEARCH ARTICLE

Performance of three small-scale wastewater treatment plants. A challenge for possible re use

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Abstract The study focused on the assessment of the performance of three WWTPs in Greece by the estimation of the microbiological and chemical quality of influent and effluent sewage. Physicochemical parameters were recorded (temperature, pH, COD, BOD, suspended solids, conductivity), and meteorological data were collected (air temperature, rain). Microbiological parameters were analyzed (*Escherichia coli*, total coliforms, bacteriophages, *Salmonella*, human adenoviruses, *Candida*, *Pseudallescheria boydii*, helminths, parasites *Cryptosporidium* ssp., and *Giardia* spp.). Statistically significant correlations among the various aforementioned parameters were investigated, in an attempt to propose appropriate processing performance indicators. Furthermore, the study aimed to assess current joint ministerial decision (JMD) on wastewater reuse, for irrigation purposes; to evaluate its practicability and its potential for public health protection. In the vast majority, outlet samples from all three studied WWTPs were not appropriate for irrigation reuse purposes based on BOD_{50} and suspended solids limit values, set by the current JMD, for both limited and unrestricted irrigation applications. Reductions for *E. coli*, total coliforms, and bacteriophages were found to range between 2–3, 1.5–2.5, and 2–4 $\log_{10}$ values, respectively. *Salmonella* spp. was detected in outlet sewage samples from Patra (PAT), Arachova (ARH), and Livadeia (LEV), at 23 % (3/13), 33 % (4/12), and 38 % (5/13), respectively. Molds were detected at 92.3 % (12/13), 100 % (13/13), and 91.6 % (11/12), respectively, while *Candida* was found at 85 % (11/13), 67 % (8/12), and 46 % (6/13). A high prevalence of *Pseudallescheria boydii*, in outlet samples from all studied WWTPs is an important public health issue, which underlines the need for further studies on this emerging fungal pathogen in wastewater reuse applications. *Pseudallescheria boydii* was found at 85 % (11/13), 67 % (8/12), and 46 % (6/13), respectively. Helminths were found in both inlet and outlet samples, of all studied WWTPs, at 100 %. Human adenoviruses, were detected at high percentages in outlet samples at 76.9 % (10/13), 92.3 % (12/13), 84.6 % (11/13), respectively, while no influence of UV irradiation was recorded on the entry and exit loads of human adenoviruses. No influence of meteorological parameters was found on the microbiological and chemical parameters, with the exception of a weak positive correlation between environmental temperature and bacteriophages. A moderate positive correlation was found between BOD and suspended solids, bacteriophages, and total coliforms, bacteriophages and *E. coli*, and bacteriophages and adenoviruses. A significant positive correlation was found between total coliforms and *E. coli*, COD and BOD, and suspended solids and COD. No correlations were proved between human pathogens and bacterial indicator parameters. Collectively, our findings

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underlined the unsuitability of the current JMD on wastewater reuse in Greece, or public health protection. The study is expected to support the development of a public health risk assessment model based on quantitative risk assessment on the use of treated wastewater for irrigation purposes in Greece.

Keywords Wastewater treatment · Sewage · Inlet · Outlet microbiological quality · Adenovirus · Risk assessment

Introduction

Wastewater presents a timely dynamic collection point where many physical, chemical, and biological substances of the society are brought to a central location (Sinclair et al. 2008).

Although, wastewater, prior entering the treatment plant contains from 100,000 to 1,000,000 microorganisms per milliliter, the majority of them are not pathogenic to humans but are significant for the biological treatment process. However, a wide variety of pathogenic organisms pass through wastewater treatment plants (WWTPs).

Urban WWTPs were originally designed to reduce the biological oxygen demand, total suspended solids (TSS), and nitrogen and phosphorus pollution, with the removal of pathogenic microorganisms receiving less attention (Lucas et al. 2014). Numerous known and unknown pathogenic microorganisms, bacteria, viruses, protozoa, fungi, and helmints, are of great concern in wastewater treatment. Pathogenic bacteria and viruses may be present at concentrations as high as 10^6 and 10^{10} per gram of wet weight of feces, respectively (Gerardi and Zimmerman 2005; Kokkinos et al. 2011). Pathogenic bacteria commonly found in wastewater include *Salmonella* (Wang et al. 2014; Espigares et al. 2006; Gerardi and Zimmerman 2005; Dumontet et al. 2001). The diversity of microbiota of wastewaters has not been thoroughly examined, despite the potential role that microbes have on the quality of effluent (Wang et al. 2014). Known and novel viruses have recently been detected for example, by metagenomic studies, making untreated wastewater the most diverse viral metagenome examined so far (Cantalupo et al. 2011).

In addition to bacterial and viral pathogens, pathogenic fungi are commonly detected in wastewater, with *Candida* being a well-known Blastomyces (Dumontet et al. 2001). Also, emerging fungal pathogens are receiving increased attention. *Pseudallescheria boydii* (anamorph *Scedosporium apiospermum*), a typical hyphomycetes, is an example of special public health importance; it has been detected in abundance in sewage sludge. *Pseudallescheria boydii* causes localized or disseminated infections called hyalohyphomycosis; shows high morphological, physiological, and molecular variability; and is also resistant to many antimycotic drugs (Janda-Ulfig et al. 2008).

Parasitic helminths that have been found in relatively large numbers in wastewater include cestodes (tapeworms) and nematodes (roundworms) (Gerardi and Zimmerman 2005). The health concern arising from the presence of zooparasites in sewage is probably underestimated, despite increased interest in parasitology. In a sewage treatment plant of a middle-sized European city, the input of parasitic helminths could easily reach several billions a day (Dumontet et al. 2001).

Microbiological monitoring of representative microbial indicators is used as a mean to detect such pollution in treated wastewater effluent (Naidoo and Olaniran 2014; Sinclair et al. 2008). The microbial removal rate of WWTPs is generally assessed using fecal bacteria as indicators (Lucas et al. 2014); however, viruses and protozoa are more resistant to removal by sewage treatment (Carducci and Verani 2013). As bacterial indicators generally fail to signal the possible viral contamination, human adenoviruses (hAdVs) have been proposed as alternative indicators. HAdVs have been shown to be excreted by the populations of all geographical areas, are the most abundant viruses detected in urban sewage without significant seasonal variation, and are also highly resistant to natural and artificial disinfection processes and procedures. For all these reasons, they have been proposed as indicators of human fecal contamination in water and food (Pina et al. 1998; Mena and Gerba 2009). Recent studies have focused on the quantification of hAdVs in wastewater samples (Fumian et al. 2013; Fong et al. 2010; La Rosa et al. 2010).

The implementation of current regulations on wastewater treatment has significantly reduced the levels of microbiological contamination of receiving waters. However, microorganisms are still widely disseminated in surface waters through discharges of untreated and treated sewage (Cantalupo et al. 2011).

Also, although the examination of associations between climatic factors and pathogen removal by WWTPs can be very important in defining the interventions and strategies needed to protect public health, very few studies focus on such associations (Lucas et al. 2014; Carducci and Verani 2013; Plósz et al. 2009).

The effluents of WWTPs receiving urban sewage may be discharged into surface or sea water or they may be reclaimed for industrial or agricultural purposes (Carducci and Verani 2013; Carducci et al. 2008). One third of the world's food is produced by irrigating about 240 million hectares out of which approximately 20 million hectares are irrigated with wastewater or sewage polluted surface water. Only 10 % of the total is being irrigated with treated wastewater (Jiménez et al. 2010). Primary and secondary treatments are able to remove up to 99 % of fecal indicator bacteria (FIB), but depending on the influent FIB concentrations, this extent of removal could be insufficient to achieve the quality required to use treated wastewaters for irrigation purposes (Lucas et al. 2014).

The risk of disease transmission from pathogenic microorganisms present in irrigation water is influenced by the level of contamination; the persistence of pathogens in water, in soil, and on crops; and the route of exposure. Human wastewater is usually of very poor microbial quality and requires extensive treatment before it can be used safely to irrigate crops (Steele and Odumeru 2004).

Primary products must not be produced in areas where water used for irrigation might constitute a health hazard to the consumer through food (Koopmans and Duizer 2004). According to the recommendations of Codex Alimentarius (FAO/WHO, 2012), water of suitable quality should be used for irrigation. In Europe, regulations (Regulation (Ec) No. 852/2004 of the European Parliament and of the Council on the Hygiene of Foodstuffs, and Article 5 (c) of Annex 1 of the General Hygiene Provisions for Primary Production and Associated Operations) state that potable water, or clean water to be used whenever necessary to prevent contamination (Maunula et al. 2013). The Council Directive 91/271/EEC concerning urban wastewater treatment was adopted on 21 May 1991. Its objective is to protect the environment from adverse effects of urban wastewater discharges and discharges from certain industrial sector and concerns the collection, treatment and discharge of domestic wastewater, mixture of wastewater, and wastewater from certain industrial sectors. Among its requirements are controls of sewage sludge disposal and reuse, and treated wastewater reuse whenever it is appropriate. According to the EU Directive 91/271/EEC subsequent national regulations (Joint Ministerial Decisions 145116/2011 and 191002/2013, regulations 145447 and 1589) are dealing with wastewater reuse issues in Greece.

This paper presents a study for the evaluation of the microbiological and chemical quality of influent and effluent sewage in three WWTPs in Greece. Physicochemical parameters were recorded (temperature, pH, COD, BOD, suspended solids, conductivity), and meteorological data were collected (air temperature, rain). Microbiological parameters were analyzed (*Escherichia coli*, total coliforms, bacteriophages, *Salmonella*, human adenoviruses, *Candida*, *Pseudallescheria boydii*, helminths, parasites *Cryptosporidium* ssp., and *Giardia* spp.). Possible correlations among the various aforementioned parameters were investigated, in an attempt to select appropriate processing performance indicators. Furthermore, the study aimed to assess the current joint ministerial decision on wastewater reuse, for irrigation purposes, to evaluate its practicability and its potential for public health protection. The novelty of the paper resides in its focus on a complete set of physical, chemical, microbial, and meteorological parameters to characterize the efficiency of treatment processes of three WWTPs in Greece, and to evaluate the reuse of wastewater for irrigation purposes and the feasibility of the relevant legislation, considering the current environmental and political contexts.

Materials and methods

Sampling

Three WWTPs have been used, each receiving sewage of populations with different caracteristics; Patras is an urban city, Levadia is a small city with intense agricultural activities, and Arachova is a tourist resort.

WWTP of Rion, Patras, was inaugurated in 1990. The volume of inffluent wastewater ranges between 1000 and 2000 m^3/day. The population equivalent is 5000, and the sewer is separated. It treats mainly urban, as well as some hospital sewage deriving from the General University Hospital of Patras. The treated liquid effluents are disposed superficially during the dry period, in an area of 20 acres with eucalyptus and poplar trees. During the wet period, the effluents are disposed in a stream finally leading to the Patraikos Gulf.

The WWTP of Livadeia was inaugurated in 1993. The mean daily volume of wastewater at the entrance is 5500 m^3/day, the average hourly flow is 400 m^3/h, and the population equivalent is 25,000. It is a combined sewerage system treating urban sewage and collecting rainwater runoff. The treated effluents are disposed in the Erkyna river. Treated effluents are reused at 10 % for industrial applications. Controlled irrigation of cropland is also performed during the irrigation season.

The WWTP of Arachova was inaugurated in 1992. The mean daily volume of wastewater at the entrance is 3000 m^3/day, the average hourly flow is 250 m^3/h, and the population equivalent is 4500. It is a combined sewerage system treating urban sewage and collecting high volumes of rainwater runoff, which contribute to the dilution of sewage. The chlorination is performed periodically, rather than constantly. The treatment process contains a sludge thickener, but no activated sludge step is performed. The treated effluents are reused for irrigation purposes of olive trees, wheat, cotton, and for garden watering, at 90–95 %. Summarized characteristics of the three WWTPs of the study are presented in Table 1.

Sampling procedure

Influent and effluent samples were collected from all three WWTPs. Ninety wastewater samples (1 and 10 L from the WWTP inlet and outlet, respectively), were collected monthly (January–December 2013), in total. Some physicochemical parameters were recorded in situ and meteorological data were recorded. Samples were transferred to the laboratories immediately, and microbiological parameters were determined. Bacterial indicators and *Salmonella* spp. were analyzed at the Technological Educational Institute of Athens (TEIA). Virological analysis was performed at the University of Patras. Mycological analysis was done at the

Table 1 Summarized characteristics of the three WWTPs of the study

	Type of treatment	Screening	Grit collector	Grease trap	Oxidation ditch	Primary sedimentation	Secondary sedimentation	Chlorination	Sludge thickener	Dewatering
PAT	Secondary	√	√	–	√	–	√	√	√	–
LEV	Secondary	√	√	√	√	√	√	√	√	√
ARH	Tertiary	√	√	–	√	√	–	√	√	–

TEIA and National Kapodistrian University of Athens. Coliphages were analyzed at the National School of Public Health (NSPH). Chemical parameters were analyzed by the ATEI and the NSPH, while sewage parasitological analysis was performed by the NSPH and the Department of Parasitology of the University of Kiel, Germany.

Microbiological and physicochemical analysis

Sewage samples were analyzed for total coliforms and *E. coli*, using the multiple tube method (CEN BT/TF 151 – PrEN 15214–3), bacteriophages, using the double-layer method (ISO 10705–2:1000), *Salmonella* spp., using an ISO method (DRAFT ISO 19250:2009 (E)), molds, *Candida*, and *Pseudallescheria boydii*, according to previously published methods (Katragkou et al. 2007; Cortez et al. 2008; Stripeli et al. 2009), helminths according to APHA standard method (2012), and *Cryptosporidium* and *Giardia*, as described by Spanakos et al. (2015).

Physicochemical parameters (COD, BOD, suspended solids, conductivity) were determined according to EPA Standard Methods for the Examination of Water and Wastewater (APHA).

Virological analysis of sewage samples for hAdV detection

Standard Operating Procedures (SOPs) of VITAL (www.eurovital.org) and VIROCLIME (www.viroclime.org) FP7 research projects of food and environmental virology were used for the virological analysis of hAdVs. The skimmed milk flocculation procedure was applied for virus concentration (Calgua et al. 2008), while the viral molecular detection was based on TaqMan assays previously described (Hernroth et al. 2002), and designed to quantify all common human AdVs with high specificity in environmental samples (Bofill-Mas et al. 2006). Viral nucleic acids were extracted from concentrated samples using the QIAamp Viral RNA minikit (Qiagen) according to the manufacturer's instructions. They were stored at − 80 °C, until the molecular virological analysis for the detection of human AdVs.

Meteorological monitoring

Meteorological data (temperature, rain) were collected from the Meteodata-base of the National Observatory of Athens (NOA), through the Meteo Search publicly accessible website. The network of automatic stations consists of stations type Davis, which measure all basic meteorological parameters, i.e., pressure, temperature, humidity, rainfall, direction, and wind strength. The meteorological data from Arachova were collected from the station "Arachova LGC5," which is located at the Arachova water reservoir; at a height of 1069 m. Meteorological data from Livadeia were collected from "Livadeia LGI8" station, which is located at the Livadeia WWTP, at a height of 120 m. Finally, data for Rion, Patras, were collected from "Patra LG84" meteorological station, which is located at the Patras old port, at a height of 6 m.

Ultraviolet (UV) (310 nm-UVB, 380 nm-UVA mW/m^2 nm), ozone monitoring instrument daily satellite data (AURA/OMI) were also provided by the NOA, after request.

Statistical analysis

Correlations of microbiological, physicochemical, and meteorological parameters were investigated for all three WWTPs of the study. SPSS 21.0 statistical software was used for the statistical analysis. Shapiro-Wilk was used for performing tests of normality for the quantitative wastewater quality parameters at the entrance of the WWTPs (p value<0.5). For the initial variables or their transformations (with whom the homogeneity of variations hypothesis is satisfied) for which the normality hypothesis is not rejected in each one of the WWTPs, ANOVA was used to investigate the hypothesis of equality of the mean values (analysis of variance by one factor–WWTP). For the rest of the variables, Kruskal-Wallis test was used. Quantitative parameters at the entrance of the WWTPs were also correlated to meteorological parameters (Spearman's test). Similarly to Carducci and Verani (2013), the plant removal rate (PRR) was calculated according to the following: PRR=entry load−exit load. Tests of normality, for PRRs, were performed by Shapiro-Wilk. In each WWTP where the PRR for a quality parameter followed the normal distribution, the one-sample test (equivalent test with paired samples t test for pairs: input, output) for the tests of hypothesis: H0: $\mu_{PRR}=0$ vs H1: $\mu_{PRR}\neq 0$, was used. As a nonparametric analogue of paired samples *t* test, in cases of lack of normality of PRRs, a sign test was used. Sign test was used for BOD at PAT, suspended solids at LEV, and conductivity at PAT and LEV. The aim was to test whether the values of the outlet variables tend to be lower than the corresponding values at the entry. After a test of homogeneity of variances, analysis of variance (ANOVA by one factor–WWTP) was used to investigate the equality of mean values for PRRs at the three WWTPs. McNemar test (significance of change of a situation) showed that the null hypothesis that the treatment does not affect the absence/presence of molds in each WWTP was rejected (at a level of significance α=0.05).

Results and discussion

Physicochemical characterization

Table 2 summarizes selected data of the physicochemical characterization of the three WWTPs of the study, with mean and standard deviation values along with maximum and minimum values per selected parameter at the inlet and outlet of each WWTP. The highest sewage temperature (30 °C) was recorded at LEV, during May, while the lowest (5 °C) at ARH, during December. At the inlet of ARH, minimum (20 mg/L) and maximum (815 mg/L) COD values were recorded during November and March, respectively. At the outlet of ARH and PAT, maximum (153 mg/L) and minimum (3 mg/L) COD values were recorded, respectively, during April and January. As it concerns BOD inlet values, the maximum value (420 mg/L) was recorded during June and July at LEV, while the minimum value (10 mg/L) was recorded during March, at ARH. BOD outlet maximum (65 mg/L) and minimum (1 mg/L) values were found during October (ARH) and November (PAT), respectively.

Note that for limited irrigation purposes, BOD_{50} limit value has been set to 25 mg/L, and that of SS at 35 mg/L, while for unrestricted irrigation applications, both BOD_{50} and SS values have to be ≤10 mg/L for 80 % of the samples (Joint Ministerial Decision 5673/400/1997).

Mean BOD_{50} values at the outlet of the WWTPs were 5.88 ±3.43, 23.42±16.48, and 23.75±19.37, for PAT, LEV, and ARH, respectively. BOD_{50} values were equal to 10 mg/L at 30.8 % (4/13) and ≤10 mg/L at 69.2 % (9/13) of the tested

Table 2 Summarized selected data of the physicochemical characterization of the three WWTPs of the study

WWTP		COD (mg/L)	BOD (mg/L)	Suspendedsolids (SS)	Conductivity (Ms/cm) 25 °C
	Mean	288	874	88	1605
PAT (inlet)	Std. deviation	127	2685	34	1027
	Minimum	90	74	30	660
	Maximum	515	9810	162	4640
PAT (outlet)	Mean	12	6	11	1257
	Std. deviation	9	3	5	450
	Minimum	3	1	3	512
	Maximum	37	10	20	1820
LEV (inlet)	Mean	410	206	207	1164
	Std. deviation	237	119	171	808
	Minimum	20	10	40	277
	Maximum	780	420	690	3380
LEV (outlet)	Mean	53	23	29	784
	Std. deviation	41	16	31	408
	Minimum	5	3	2	172
	Maximum	110	42	107	1260
ARH (inlet)	Mean	363	182	165	744
	Std. deviation	235	118	91	321
	Minimum	32	17	34	278
	Maximum	815	366	360	1196
ARH (outlet)	Mean	55	24	23	592
	Std. deviation	50	19	16	331
	Minimum	6	5	4	132
	Maximum	153	65	55	1080

Mean and standard deviation values are shown along with maximum and minimum values per selected parameter at the inlet and outlet of each WWTP outlet samples from PAT. Outlet samples from LEV were found with BOD_{50} values ≤10 mg/L at 38.5 % (5/13), while 53.8 % (7/13) were found with BOD_{50} values ≤25 mg/L. Similarly, 33.3 % (4/12) outlet samples from ARH were found with values ≤10 mg/L, while 58.3 % (7/12) were ≤25 mg/L.

Mean SS values at the outlet of the WWTPs were 5.88± 3.43, 23.42±16.48, and 23.75±19.37, for PAT, LEV, and ARH, respectively. Outlet samples with SS values ≤25 mg/L were 100 % (13/13), 53.8 % (7/13), and 58.3 % (7/12), for PAT, LEV, and ARH, respectively, while samples with SS values ≤10 mg/L were 61.5 % (8/13), 30.8 % (4/13), and 33.3 % (4/12).

According to the afforementioned results in the vast majority of the cases, outlet samples are not appropriate for irrigation reuse purposes based on BOD_{50} and SS limit values.

Microbiological analysis

In inlet and outlet sewage samples from PAT, LEV, and ARH, bacterial indicators (*E. coli* and total coliforms), and bacteriophages were determined ($\log_{10}$ of mean values) and are summarized in Table 3. Although precise comparisons are difficult because of differences in sewage concentrations, local climate conditions and plant technologies, the average entry and exit loads and removal rates were consistent with previous surveys (Carducci and Verani 2013; Petrinca et al. 2009). Reductions ($\log_{10}$ values) for *E. coli*, total coliforms, and bacteriophages were found to range between 2–3, 1.5–2.5, and 2–4, respectively.

Although wastewater is treated to eliminate pathogenic microorganisms and prevent waterborne transmission, numerous studies indicate that conventional wastewater treatment does not guarantee their complete elimination. *Salmonella* is known to survive despite of treatment and its transmission through wastewater is a public health issue of particular importance (Espigares et al. 2006). *Salmonella* spp. was detected in inlet sewage samples of PAT, ARH, and LEV, at 31 % (4/13), 50 % (6/12), and 62 % (8/13), respectively, while in outlet samples, it was found at 23 % (3/13), 33 % (4/12), and 38 % (5/13), respectively.

Table 3 Summarized results of microbiological analyses regarding the parameters of *E. coli*, total coliforms (TC), bacteriophages, and adenoviruses (hAdVs), at the inlet (n=13) and outlet (n=13) of each of the three WWTPs of the study (mean values±SD)

	E.coli	TC	Bacteriophages	hAdVs
PAT (inlet)	4.96	5.66	1761	2.49 (±1.49)
PAT (outlet)	3.38	3.78	49	2.83 (±1.74)
LEV (inlet)	6.53	7.06	8565	3.93 (±0.76)
LEV (outlet)	4.03	4.45	659	3.97 (±1.41)
ARH (inlet)	6.06	6.26	5911	3.14 (±1.81)
ARH (outlet)	3.95	4.48	417	3.35 (±1.68)

For *Cryptosporidium* and *Giardia* spp., please refer to Spanakos et al. (2015); units: *E. coli*, $\log_{10}$ MPN/L; TC, $\log_{10}$ MPN/L; bacteriophages, $\log_{10}$ plaque forming units (PFU)/L; hAdVs, $\log_{10}$ GC/L

Molds were detected at 92.3 % (12/13), 100 % (13/13), and 91.6 % (11/12) in outlet sewage samples from PAT, LEV, and ARH, respectively. *Candida* was detected in inlet sewage samples of PAT, ARH, and LEV at 54 % (7/13), 83 % (10/12), and 62 % (8/13), respectively, while in outlet samples, it was found at 85 % (11/13), 67 % (8/12), and 46 % (6/13), respectively. *Pseudallescheria boydii* is an emerging fungal pathogen. The highest densities of *Pseudallescheria boydii*/*Scedosporium apiospermum* complex (PSC) isolates were recently reported in human-impacted areas, including fluids obtained from wastewater treatment plants (Rougeron et al. 2014). The highest percentage of positives for *Pseudallescheria boydii* in inlet samples was found at LEV (54 %, 7/13), followed by ARH (50 %, 6/12) and PAT (31 %, 4/13). Similarly, the highest percentage of positives in outlet samples, was found at LEV (38 %, 5/13), followed by PAT (31 %, 4/13) and ARH (25 %, 3/12). The high prevalence of *Pseudallescheria boydii* in outlet samples from all studied WWTPs is an important public health issue, which underlines the need for further studies on this emerging pathogen in wastewater reuse applications.

Cryptosporidium spp. and *Giardia lamblia* (synonyms: *Giardia duodenalis*, *Giardia intestinalis*) are emerging protozoa causing disease in humans and animals worldwide. One of the sources of these parasites can be treated wastewater from wastewater treatment plants (Hachich et al. 2013; Sroka et al. 2013). In an attempt to enrich poor existing data on the prevalence of these parasites in wastewater effluents in Greece, the study produced very interesting data from the parasitological analysis of the sewage samples of the study (Spanakos et al. 2015).

Activated sludge systems demonstrate average removal rates of 92 and 87 % for parasite eggs and cysts, respectively, which indicates the possibility of survival of protozoan (oo)cysts and helminth eggs beyond typical retention times for treatment processes (Sroka et al. 2013). In the present study, helminths were found in both inlet and outlet samples, of all studied WWTPs, at 100 %.

Human adenoviruses were detected at 76.9 % (10/13), 100 % (13/13), and 76.9 % (10/13) in inlet sewage samples from PAT, LEV, and ARH, respectively, while they were also found at high percentages in outlet samples at 76.9 % (10/13), 92.3 % (12/13), and 84.6 % (11/13), respectively. Inlet and outlet hAdV concentrations for the three WWTPs are shown in Table 3. No influence of UV irradiation was found on the entry and exit loads of human adenoviruses. Carducci and Verani showed the presence of adenovirus DNA in 100 % of collected samples, while an average reduction of 2 log was recorded (Carducci and Verani 2013). Similarly, AdVs were detected in 100 % of wastewater and sewer overflows (CSOs) discharge samples in the study of Fong et al. (2010). Average adenovirus DNA concentrations in sewage and CSOs were 1.15×10(6) viruses/liter and 5.35×10(5) viruses/liter, respectively, while the adenovirus removal was calculated at <2 log(10) (99 %) for this WWTP (Fong et al. 2010). AdVs ranged from 4.6×10(4) to 1.2×10(6) and from 50 to 1.3×10(4) GCs/ml in sludge and wastewater, respectively, in the study of Schlindwein et al. (2010). Viral viability analyses by cell culture (ICC-PCR) resulted in high viability of AdVs in sludge (100 %) and in wastewater (66.6 %). In a recent study, hAdVs were the most prevalent viruses found in influent samples (100 %) with a virus load that ranged from 10(6) to 10(5) genome copies per liter (GCs l(−1)) (Fumian et al. 2013). Although AdVs were detected in 7.41 %, 3.45 %, and 2.78 % of the samples collected in three sampling periods from a WWTP, they were never detected in two other WWTPs in the study of Petrinca et al. (2009). Adenoviruses were also detected in 96 % of inlet and 94 % of outlet samples, supporting the potential of these viruses as indicators of viral contamination from sewage by Myrmel et al. (2006). An association between meteorological parameters and viral removal rates was detected only for rainfall and hAdV removal during a specific sampling campaign, suggesting that hAdV is a suitable parameter to assess the viral removal efficiency of wastewater treatment plants, particularly in the case of heavy rainfall (Carducci and Verani 2013). No physicochemical, meteorological, or bacterial indicators correlated with hAdV loads and reduction, confirming the poor utility of these organisms as indicators of viral removal. The high viral load in sewage results in a discharge to the environment of a large amount of virus despite sewage treatment (Myrmel et al. 2006). A better understanding of viral presence and resistance to sewage purification processes have the potential of contributing to the effective management of risks linked to the recycling of treated wastewater, and its discharge into the environment (La Rosa et al. 2010).

In an attempt to compare the quality of raw wastewaters at the inlet of the three WWTPs of the study, the mean values of different parameters were compared (ANOVA or Kruskal-Wallis). Statistically significant differences of the mean values ($p<0.05$) of the following parameters were observed, and their trend is presented for the three WWTPs: *E. coli*: PAT<LEV, ARH; total coliforms: PAT<LEV; suspended solids: PAT<LEV; conductivity: PAT>ARH; bacteriophages: PAT<LEV, ARH; adenoviruses: PAT<LEV, ARH. The WWTP of Patras receives raw wastewaters of higher quality according to the studied parameters.

Collectively, no influence of meteorological parameters was found on the microbiological and chemical parameters of the raw wastewater at the inlet of the studied WWTPs, with the exception of a weak positive correlation between environmental temperature and bacteriophages (Spearman's rho 0.386, $p<0.05$). A weak positive correlation was found between bacteriophages and total coliforms (Spearman's rho 0.430, $p<0.01$) and between bacteriophages and *E. coli* (Spearman's rho 0.393, $p<0.05$). A moderate positive correlation was found between BOD and suspended solids (Spearman's rho 0.684, $p<0.01$). A significant positive correlation was found between total coliforms and *E. coli* (Spearman's rho 0.762, $p<0.01$), COD and BOD (Spearman's rho 0.761, $p<0.01$), and suspended solids and COD (Spearman's rho 0.807, $p<0.01$). No correlation was found between human pathogens and bacterial indicator parameters. The microbial removal rate of WWTPs is generally assessed using fecal bacteria (e.g., *E. coli* and enterococci) as indicators; however, viruses and protozoa are more resistant to removal by sewage treatment and the literature is continuously enriched with data on the limitations of using classic microbiological indicators as the sole microbiological criterion of the occurrence and concentration of pathogens (Spanakos et al. 2015; Carducci and Verani 2013; Cook 2013). Regarding the PRR, strong correlations were found between COD and BOD (Spearman's rho 0.752, $p<0.01$) and between COD and suspended solids (Spearman's rho 0.720, $p<0.01$). Moderate correlations were found between *E. coli* and total coliforms (Spearman's rho 0.668, $p<0.01$), bacteriophages and air temperature (Spearman's rho 0.499, $p<0.01$), and between suspended solids and BOD (Spearman's rho 0.636, $p<0.01$). Weak correlations were also found between bacteriophages and conductivity (Spearman's rho 0.383), BOD and air temperature (Spearman's rho 0.318), BOD and rain (Spearman's rho 0.304), and COD and rain (Spearman's rho 0.309).

In an attempt to evaluate the adequacy and efficiency of the current joint ministerial decision on wastewater reuse in Greece, for public health protection, our findings interestingly underlined its unsuitability, concerning all its referred parameters. Any revisions of the guidelines and any proposal for the use of other/different quality criteria needs an extensive risk assessment analysis, using real datasets, as the ones collected in the context of the present study. The performance of each one of the studied WWTPs (as it concerned microbiological, chemical parameters, etc.), after all treatment procedures has been proved insufficient, with the overall quality of the produced treated effluents being low for reuse purposes.

Secondary treatment does not provide an outlet product consistently to the regulation's standard and so low quality effluent is disposed, but chlorination did not have a considerable effect. Sewage-related microorganisms demonstrate different sensitivities to chlorination and these patterns of sensitivity are dependent on the species of chlorine present, the dose of chlorine applied, and the way that the target organism is presented to the disinfectant. Bacterial indicators may not be representative of the behavior of human enteric viruses and parasites, following wastewater disinfection (Spanakos et al. 2015; Tree et al. 2003). Especially when BOD values stay high after treatment, chlorine disinfection effect is low, since chlorine residuals are unstable in the presence of high concentrations of chlorine-demanding materials.

The real quality of the treated effluents is not in accordance to the new legislation for wastewater reuse and consequently treated effluents are not appropriate for irrigation purposes. Although a statistically significant reduction of the concentration of *E. coli*, TC, and bacteriophages at the outlet compared to the inlet of the studied WWTPs was recorded, the concentrations of the aforementioned microbiological parameters still remain high. The following microbiological parameters: *Cryptosporidium*, *Candida* spp., *Pseudallescheria boydii*, and adenoviruses, at the inlet, cannot be accurately measured, since there are currently no ISO methods for the detection of parasites, fungi, and viruses in sewage, as it is the case for instance for bacterial indicators. These parameters are not suitable indicators of performance of the WWTPs as their detection and monitoring is still laborious and expensive and cannot be used in a routine laboratory.

According to this study, bacteriophages seem to be suitable as performance indicators. They have already been proposed as potential performance indicators for the easier environment of mains water treatment plants. Their presence correlate well with bacterial fecal indicators, the method is simple, and it does not seem to be affected by sewage environment. It provides constantly a clear difference between inlet and outlet sewage, and once applied in a routine laboratory, it shows high repeatability. As for the applied method for viruses, it is better performed than the one for parasites. Nevertheless, viruses are not indicated as a suitable performance indicator, as the secondary treatment in WWTPs does not have a considerable effect on viruses.

A prerequisite of wastewater reuse is the selection and establishment of appropriate quantitative indicators; it has been proven that no indicator ensures safety of reuse considering the complete set of parameters since there is no correlation between them. Moreover, a systematic study of quality parameters is required. Analytical laboratories should assure that there are analytical methods for the parameters or contaminants of interest, and that they are especially appropriate for wastewater, since there are no ISO methods for sewage analysis, except for bacterial indicators. Although the reuse of treated effluents for irrigation purposes can make a significant contribution to the integrated management of our water resources, it has to be underlined that nonappropriately managed effluents can pose significant environmental, public health or agricultural resource risks. Risk assessment studies are effective tools toward wastewater reuse in different applications, including irrigation. Modernization of the wastewater collection and treatment along with the reduction of treatment costs should be supported. Separation at home of gray, yellow, and black waters should be promoted (Gaulke 2006).

The study is expected to support the development of a public health risk assessment model on the use of treated wastewater for irrigation purposes. It will provide public health scientists, authorities, environmentalists, and other people dealing with wastewater reuse in Greece the opportunity to discuss the issues of effluent quality and the reuse potential.

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