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Peracetic Acid (PAA) Disinfection: Inactivation of Microbial Indicators and Pathogenic Bacteria in a Municipal Wastewater Plant

Silvia Bonetta [1,*], Cristina Pignata [1], Eugenio Lorenzi [2], Margherita De Ceglia [2], Lorenza Meucci [2], Sara Bonetta [1], Giorgio Gilli [1] and Elisabetta Carraro [1]

[1] Department of Public Health and Pediatrics, University of Torino, Piazza Polonia 94, 10126 Torino, Italy; cristina.pignata@unito.it (C.P.); sara.bonetta@unito.it (S.B.); giorgio.gilli@unito.it (G.G.); elisabetta.carraro@unito.it (E.C.)

[2] Società Metropolitana Acque Torino S.p.A., C.so XI Febbraio, 14, 10152 Torino, Italy; eugenio.lorenzi@smatorino.it (E.L.); margherita.deceglia@smatorino.it (M.D.C.); lorenza.meucci@smatorino.it (L.M.)

* Correspondence: silvia.bonetta@unito.it; Tel.: +39-011-670-5821

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Abstract: Several studies have noted that treated and untreated wastewaters are primary contributors of a variety of pathogenic microorganisms to the aquatic ecosystem. Conventional wastewater treatment may not be sufficient to achieve microbiologically safe effluent to be discharged into natural waters or reused, thus requiring wastewater effluents to be disinfected. In recent years, peracetic acid (PAA) has been adopted as a disinfectant for wastewater effluents. The aim of this study was to evaluate the disinfection efficiency of PAA at low doses (range 0.99–2.10 mg/L) against microbial indicators and pathogenic bacteria in a municipal wastewater plant. Samples of untreated sewage and effluents before and after PAA treatment were collected seasonally for 1 year and were analysed for pathogenic *Campylobacter*, *Salmonella* spp., *E. coli* O157:H7 and *E. coli* virulence genes using molecular methods; moreover, the detection of specific microbial indicators (*E. coli*, faecal coliforms, enterococci, *C. perfringens*) and *Salmonella* spp. were carried out using culturing methods. *Salmonella* spp. DNA was found in all untreated sewage and effluent before PAA treatment, whereas it was recovered in 50% of the samples collected after PAA treatment. Although *E. coli* O157:H7 was never identified, the occurrence of Shiga-like toxin I amplicons was identified in 75% of the untreated sewage samples, in 50% of the effluents assayed before PAA treatment, and in 25% of the effluents assayed after PAA treatment, whereas the *stx2* gene was never found. *Campylobacter coli* was only detected in one effluent sample before PAA treatment. In the effluents after PAA treatment, a lower load of indicator bacteria was observed compared to the effluents before treatment. The results of this study highlight that the use of low doses of PAA seems to lead to an improvement of the microbiological quality of the effluent, although it is not sufficient to guarantee its suitability for irrigation. These results underscore the need for additional studies to further assess the efficiency of PAA disinfection in municipal wastewater plants.

Keywords: wastewater; peracetic acid; microbial indicators; pathogenic bacteria

1. Introduction

Several studies have noted that treated and untreated wastewaters are primary contributors of a variety of pathogenic microorganisms, pollutants, and chemicals to the aquatic ecosystem [1,2]. Numerous studies have indicated that primary and secondary wastewater treatment typically achieves 90–99% reductions of enteric microbial numbers. However, this treatment may not be sufficient to

produce microbiologically safe effluent that can be discharged into natural waters [3,4]. In fact, different pathogenic and opportunistic bacteria have been found in treated effluent, including Shiga toxin (Stx) *Escherichia coli* (STEC or VTEC), *Salmonella* spp., *Campylobacter jejuni*, *Bacillus cereus*, *Clostridium difficile*, and *Listeria monocytogenes* [5,6]. The microbiological quality of wastewater can pose a number of potential risks in terms of public health and environmental contamination when also considering the possible reuse of wastewater effluents [7]. To achieve more efficient microbial elimination, a further treatment such as disinfection is therefore necessary.

Chlorine is the most widely employed disinfectant to treat wastewater before it is discharged into receiving water bodies around the world. This is because it is a widely known technology, it is low cost and it has proven efficiency in inactivating a great variety of pathogenic microorganisms [8]. However, the awareness of harmful by-products and the formation of chlorination-resistant bacteria strains has caused wastewater plants to consider other options [9]. The main alternatives to chlorination are ozonization, the use of ultraviolet light, and peracetic acid (PAA).

Commercially available PAA consists of a quaternary mixture of acetic acid, hydrogen peroxide, peracetic acid and water. It has strong oxidizing properties, and it is active against enteric bacteria and to a lesser degree against viruses, bacterial spores, and protozoan cysts [10,11]. One of the main advantages of PAA is the possibility of an easy retrofit for sodium hypochlorite disinfection equipment, which is generally present in existing wastewater treatment plants (WWTPs), thus avoiding expensive and structural interventions. This benefit has particularly favoured the spread of PAA disinfection technologies [12].

Although PAA is believed to decompose into harmless products and to form little or no by-products that are toxic or mutagenic, studies of the effects on potential effluent toxicity in secondary effluent are controversial [10,13]. However, it is generally accepted that when it is used in low doses, PAA does not generate significant amounts of toxic or mutagenic by-products, or chemical residues, in effluents [11]. The addition of low doses of PAA has various notable benefits. In addition to lowering the costs of purification, it does not lead to an increase in organic matter. Moreover, it helps to reduce the risks associated with the storage of peracetic acid during the summer period [12].

The aim of this study was to evaluate the disinfection efficiency of PAA at low doses against typical bacterial indicators and the most important zoonotic bacterial pathogens (*Salmonella* spp., pathogenic *Campylobacter*, and VTEC) in a full-scale municipal wastewater plant.

2. Materials and Methods

2.1. Bacterial Strains and Culture Media

C. jejuni (ATCC 33291), *E. coli* O157:H7 (NCTC 129, non-toxigenic strain, encoding the *eae* gene), and *S. typhimurium* (ATCC 14028) were used as quality control strains throughout this study. The *C. jejuni* strain was cultivated on blood-free Campylobacter medium base (Karmali; Biolife, Milan, Italy) or Bolton broth (Oxoid, Cambridge, UK) at 42 °C under a microaerobic atmosphere (Campygen; Oxoid, Cambridge, UK), and *E. coli* O157:H7 and *S. typhimurium* were grown on tryptic soy agar (TSA; Applichem, Darmstadt, Germany) or in tryptic soy broth (TSB; Applichem, Darmstadt, Germany) at 37 °C.

2.2. Sampling

A summary of the study design is reported in Table 1. Untreated sewage and effluents before and after PAA treatment were collected from an Italian wastewater treatment plant located in the Piedmont region. The WWTP (untreated sewage corresponding to a population equivalent of 60,000) employs screening, aerated grit removal, biological treatment (denitrification process associated with oxidation/nitrification step, sludge age: 20 days), secondary settling, and the effluent is finally disinfected with PAA (15% *w*/*w*) before being discharged into the receiving water bodies. The disinfection contact time was calculated to be approx. 30 min. The hydraulic retention time of the total plant was 3.3 h.

Table 1. Physicochemical characteristics of the raw sewage (I) and disinfected effluent (DE).

Season	Sample	pH	TSS mg/L	BOD mg/L	COD mg/L	NH_4^+ mg/L	NO_2^- mg/L N	NO_3^- mg/L N	N Total mg/L	P Total mg/L
Summer	I	7.7	122	162	341	34.8	<0.025	<1.30	32.4	4.36
	DE	7.8	<5	<5	<15	3.2	0.084	<1.30	4.3	0.62
Autumn	I	7.8	144	206	550	44.8	<0.025	<1.30	45.0	6.47
	DE	7.9	<5	<5	<15	<2	<0.025	2.69	3.1	0.25
Winter	I	7.2	71	45	320	38.4	0.067	<1.30	36.9	4.93
	DE	7.4	<5	<5	<15	<2	0.29	3.43	4.4	0.31
Spring	I	7.5	105	97	295	30.1	<0.025	<1.30	25.8	3.07
	DE	7.5	10.9	<5	22.9	<2	0.037	1.6	3.9	0.36

Notes: I = influent, DE = disinfected effluent. TSS = total suspended solids, BOD = biological oxygen demand, COD = chemical oxygen demand.

Wastewater composite samples (24 h) were collected during four sampling periods in sterile plastic bottles, transported on ice to the laboratory and tested within 24 h. Table 2 lists the physicochemical composition of the raw sewage and disinfected effluents.

Table 2. Overview of study design.

Sampling Period	Sampling Points	Cultural Analysis	Molecular Analysis	PAA Dose (mg/L)
Summer–July 2015	I, E, DE	*E.coli*, Faecal Coliforms (FC), Enterococci, *C. perfringens*, *Salmonella* spp.	*E.coli* O157:H7, Shiga toxin I, Shiga toxin II, Intimin, *Campylobacter* spp., *Campylobacter coli*, *Campylobacter jejuni*, *Salmonella* spp.	0.99
Autumn–November 2015	I, E, DE			1.10
Winter–January 2016	I, E, DE			1.06
Spring–April 2016	I, E, DE			2.10

Notes: I = influent, E = effluent, DE = disinfected effluent, PAA = peracetic acid.

2.3. Microbiological Analyses for Pathogen Detection

Samples of raw sewage (100 mL) and effluents before and after PAA treatment (1 L) were used for pathogen detection using a PCR protocol. During each sampling, a raw sewage sample that was spiked with a high concentration of pathogens (~10^6 CFU/100 mL) was prepared (positive control). The primary steps of the protocol for pathogen detection in wastewater samples have previously been reported [6]. In brief, a wastewater sample was concentrated by filtration through 0.45-µm pore size nitrocellulose filters (Merck Millipore, Vimodrone, Italy). The filters were then vortexed in peptone water (Oxoid) for *E. coli* O157:H7 and *Salmonella* spp. detection and in Bolton broth containing an antibiotic supplement (Oxoid) for *Campylobacter* spp. detection. These broths were cultivated (enrichment step) at 37 °C for 18 h for *E. coli* O157:H7 and *Salmonella* spp. detection, and at 42 °C for 48 h under a microaerobic atmosphere for *Campylobacter* spp. detection. Following incubation, 2 mL of each broth was centrifuged at 4,500× *g* for 20 min to recover the bacteria. The DNA was extracted and purified with a PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's protocol. The resulting DNA was used for PCR amplification. Details about the PCR analysis for *E. coli* O157 gene, H7 gene, the *E. coli* virulence genes (*stx1*, *stx2*, *eae*), *Salmonella* spp., and pathogenic *Campylobacter* were previously reported [6–15].

Salmonella spp. were also monitored using the culture method because this parameter is used to evaluate the possible reuse of wastewater effluent for irrigation. For *Salmonella* spp. detection, 100 mL of influent sample and 1 L of effluent sample were filtered through 0.45-µm pore size (47 mm diameter) nitrocellulose membranes (Merck Millipore) followed by a pre-enrichment step (Peptone Water, Oxoid), a selective enrichment step (Rappaport Vassililadis Broth, Oxoid), and selection on XLD Agar (Oxoid) [16]. Bacterial colonies with a typical *Salmonella* morphology were subcultured onto TSA for 18–24 h, tested for oxidase, and then identified with an API® 20E identification kit (BioMerieux, Marcy L'Etoile, France).

2.4. Microbiological Analyses for the Detection of Microbial Indicators

E. coli, enterococci, *Clostridium perfringens* spores, coliforms, and *Salmonella* spp. were analysed in all samples. In brief, the membrane filtration method was used to process wastewater samples for *C. perfringens* enumeration as reported by the ISO 14189:2013. Wastewater samples were assayed for *E. coli*, coliforms, and enterococci with a commercial Quanti-Tray^{TM} 2000 (IDEXX Laboratories, Milan, Italy) [16,17].

2.5. Statistical Analyses

Statistical analyses were performed with the SPSS Package version 22.0 for Windows. The ANOVA test and Tukey post hoc analysis were performed to evaluate the effectiveness of PAA treatment on the indicators counts. The relationship between the PAA concentration and the reduction of indicators was evaluated by Spearman's correlation.

3. Results and Discussion

The results of the molecular analyses performed for the full-scale municipal wastewater plant are reported in Table 3. *Salmonella* spp. were present in all of the raw sewage samples that were analysed using molecular methods according to the data obtained in other studies [18–20]. The presence of *Salmonella* in all influent samples underscores that the presence of pathogens in wastewater is a function of the infections that spread in the community from which the waste materials are derived [1]. Moreover, the *Salmonella* spp. contamination was observed in the effluents before PAA treatment (100%, 4/4). This finding highlights that conventional municipal wastewater treatment cannot eliminate *Salmonella* contamination, and that without efficient tertiary treatment this contamination may pose a risk to public health [18]. The detection of *Salmonella* spp. in 50% of samples (2/4) collected after PAA disinfection underscores that the low doses of PAA used (0.99 mg/L and 1.06 mg/L) were likely not sufficient to remove *Salmonella* contamination in the final effluent. A total reduction of *Salmonella* (100%) was obtained by Pradhan et al. [4] after wastewater disinfection by using a higher PAA concentration (3 mg/L). Another interesting study reported the presence of *Salmonella* in 28% of PAA disinfected effluents monitored in nine WWTPs along the coast of Venice province (Italy), but the authors did not report the values of the PAA doses used [21]. A poor efficiency of low doses of PAA (1–2 mg/L) against *Salmonella enteritidis* was also reported by Koivunen et al. [22] in laboratory-scale experiments.

Table 3. Detection of *Salmonella* spp., *E. coli* O157:H7, *E. coli* virulence genes, and *Campylobacter* (spp., *coli*, and *jejuni*) by the PCR method in a full-scale municipal wastewater treatment plant.

Salmonella				*E. coli* O157:H7			*Campylobacter*		
	Genus	**O157**	**H7**	**Intimin (*eae*)**	**SLT-I (*stx1*)**	**SLT-II (*stx2*)**	**Genus**	***C. jejuni***	***C. coli***
Summer									
I	+	−	+	−	+	−	−	−	−
E	+	−	+	−	−	−	−	−	−
DE	+	−	+	−	−	−	−	−	−
Autumn									
I	+	−	+	−	+	−	−	−	−
E	+	−	+	−	+	−	+	−	+
DE	−	−	+	+	−	−	−	−	−
Winter									
I	+	−	+	−	+	−	−	−	−
E	+	−	+	−	+	−	−	−	−
DE	+	−	+	−	+	−	−	−	−
Spring									
I	+	−	+	−	−	−	−	−	−
E	+	−	+	−	−	−	−	−	−
DE	−	−	+	−	−	−	−	−	−

Notes: I = influent, E = effluent, DE = disinfected effluent.

The results of the PCR analyses showed that 100% (12/12) of the wastewater samples were positive for H7 DNA, whereas *E. coli* O157:H7 DNA and the *stx2* gene were not detected in any of the examined samples. A total of three influent (3/4 or 75%) and two effluent (2/4 or 50%) samples revealed the presence of amplicons corresponding to Shiga-like toxin I, in agreement with the results obtained in other studies [23,24]. Otherwise, this amplicon was recovered in only one sample of effluent treated by PAA (1/4 or 25%), thus underscoring the possible activity of this disinfectant in reducing the potential health hazard associated with the presence of the *stx* genes. In fact, the *stx1*/*stx2* genes are widely distributed among *E. coli* (Shiga-toxin-producing *E. coli* or verotoxin-producing *E. coli*) and *Shigella* strains and among other waterborne bacteria because of their dissemination via bacteriophages [25,26]. However, the presence of *stx* genes is essential but not sufficient to cause infection because other major virulence factors (e.g., the *eae* gene) could play an important role [27]. In our study, the *eae* gene (related to intimin expression) was observed in only one sample of disinfected effluent, in which the *stx1* gene was absent.

Campylobacter was found in only one sample of effluent before PAA treatment, and was identified as the species *Campylobacter coli*, but no pathogenic *Campylobacter* was recovered in the samples after PAA treatment. A higher contamination of pathogenic *Campylobacter* was previously reported in the untreated sewage of the same WWTP (100% for genus, 50% for *C. jejuni*, and 50% for *C. coli*) and in effluents that had not been disinfected with PAA (50% for genus, 25% for *C. jejuni*, and 25% for *C. coli*) [6]. Considering the low frequency of contamination by pathogenic *Campylobacter*, no assessments about the effectiveness of PAA disinfection against this bacterium can be carried out.

Some classic faecal indicators (e.g., *E. coli*, coliforms, enterococci, and *C. perfringens* spores) were also analysed to verify the effectiveness of PAA disinfection and evaluate the relationship between their concentrations and the presence of pathogens. The mean reduction values of the faecal indicators at specific PAA doses are reported in Figure 1.

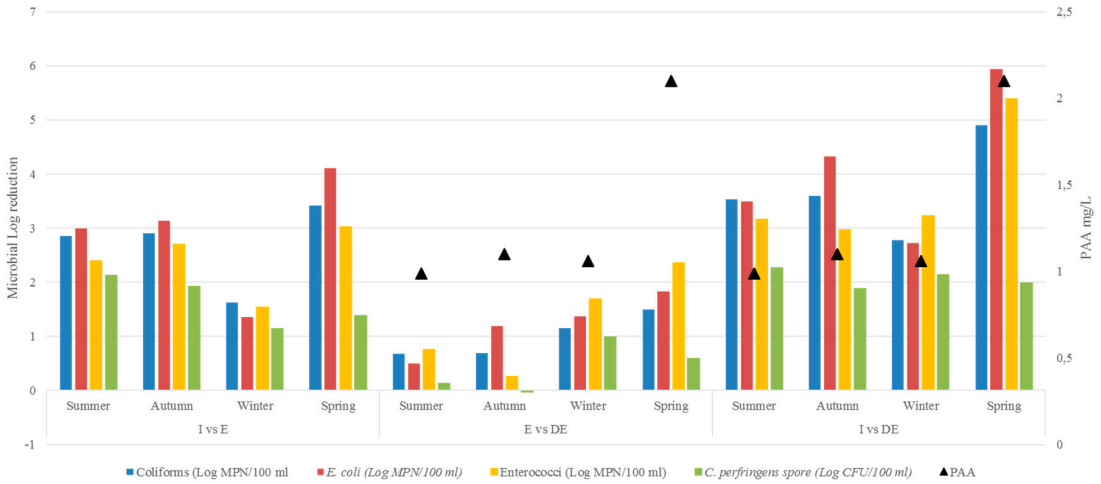

Figure 1. Log reduction of microbial indicators (expressed as log CFU/100 mL or log MPN/100 mL) in the three sampling points (influent = I, effluent = E, disinfected effluent = DE) and PAA doses tested.

During the monitoring, high concentrations [2,28] of faecal coliforms and *E. coli*, as well as enterococci and *C. perfringens* were observed in raw sewage (Table 4). The concentrations of the four bacterial indicators were similar in the summer, autumn, and spring (range: 2.91 to 4.42 log MPN/100 mL or CFU/100 mL), whereas a major contamination was observed during the winter (range: 4.21 to 5.74 log CFU-MPN/100 mL or CFU/100 mL). The concentrations of faecal coliforms in the PAA disinfected effluents was always found in a range of 3–4 log MPN/100 mL, except in the spring, when a lower concentration (2 log MPN/100 mL) was observed. Even the bacterial load of *E. coli* detected after PAA disinfection was lower in spring (~1 log MPN/100 mL) than in the other seasons (2–4 log

MPN/100 mL). The same trend was also observed for enterococci (<1 log MPN/100 mL in the spring; 2–3 log MPN/100 mL in the other three seasons). The concentration of *C. perfringens* spores was fairly constant in the disinfected effluents during the four seasons examined. It should be noted that in spring sampling, the highest concentration of PAA (2.1 mg/L) was used, which could likely be the reason for the lower concentrations of the faecal indicators observed. The different trend presented by *C. perfringens* could be explained by the higher resistance of the spores to PAA treatment, as reported by Gehr and collaborators [29].

Table 4. Concentrations of microbial indicators in the three sampling points.

Season	Sample	Faecal Coliforms MPN/100 mL	*E. coli* MPN/100 mL	Enterococci MPN/100 mL	*C. perfringens* Spore CFU/100 mL	*Salmonella* spp.
Summer	I	9.66×10^6	7.91×10^6	1.09×10^6	2.61×10^5	+
	E	1.35×10^4	8.00×10^3	4.36×10^3	1.92×10^3	+
	DE	2.84×10^3	2.52×10^3	7.56×10^2	1.41×10^3	–
Autumn	I	2.10×10^7	8.63×10^6	7.95×10^5	1.42×10^5	+
	E	2.64×10^4	6.27×10^3	1.55×10^3	1.68×10^3	+
	DE	5.42×10^3	4.09×10^2	8.45×10^2	1.84×10^3	–
Winter	I	2.33×10^7	5.93×10^6	2.17×10^6	2.26×10^5	+
	E	5.48×10^5	2.61×10^5	6.17×10^4	1.61×10^4	+
	DE	3.87×10^4	1.10×10^4	1.26×10^3	1.63×10^3	+
Spring	I	2.37×10^7	1.05×10^7	1.28×10^6	1.77×10^5	+
	E	9.20×10^3	8.23×10^2	1.21×10^3	7.30×10^3	+
	DE	2.95×10^2	1.21×10^1	5.20	1.84×10^3	–

Notes: I = influent, E = effluent, DE = disinfected effluent.

The analysis of variance (ANOVA) showed statistically significant differences between the averages of the four indicators in the different sampling points ($p < 0.05$), in particular between the influent and the effluent and between the influent and the disinfected effluent, as highlighted by the post hoc Tukey test (Table 5). No statistically significant differences were observed between the concentrations of the indicators in effluents before and after PAA treatment, probably due to the increased reduction of the bacterial load in the spring sample, when the PAA dose was doubled and to the high bacterial abatement in the pretreatment effluent. Therefore, additional sampling could be useful for confirming the obtained data regarding the higher dose of PAA tested.

Table 5. Results of the analysis of variance (ANOVA) and post hoc Tukey test.

Indicators	ANOVA *p*		Mean Abatement (Log)	Post-Hoc *p*
Faecal coliform	0.0005	I *vs* E	2.670	0.001
		I *vs* DE	3.701	0.0005
		E *vs* DE	1.001	0.158
E. coli	0.001	I *vs* E	2.899	0.005
		I *vs* DE	4.123	0.0005
		E *vs* DE	1.224	0.218
Enterococci	0.0005	I *vs* E	2.420	0.005
		I *vs* DE	3.690	0.0005
		E *vs* DE	1.270	0.117
***C. perfringens* (spore)**	0.0005	I *vs* E	1.648	0.0005
		I *vs* DE	2.070	0.0005
		E *vs* DE	0.422	0.142

Notes: I = influent, E = effluent, DE = disinfected effluent.

For compliance with the legal limit for *E. coli* counts (≤11,000 CFU/100 mL) authorized by the Province of Torino [30] for wastewaters discharged of this WWTP into surface water, the application of

PAA brought all the samples into compliance with the microbiological limits, even if they were below the limit also in the non-disinfected effluent in July, November, and April (Table 6).

Table 6. Frequency (%) of compliance of samples with Italian limits and International Guidelines for the re-use in irrigation and discharge into surface waters.

	Effluent Before PAA Disinfection	PAA-Treated Effluents	Reference
Irrigation reuse			
Italy, Ministry Decree 2 May 2006 [a]			[31]
% of sample <10 *E. coli*/100 mL	0 (0/4)	0 (0/4)	
% of sample <100 *E. coli*/100 mL	0 (0/4)	25 (1/4)	
% of sample *Salmonella* absent	0 (0/4)	75 (3/4)	
WHO, 2006			[32]
% of sample <1000 FC/100 mL	0 (0/4)	0 (0/4)	
EPA, 2012			[33]
% of sample with 0 FC/100 mL	0 (0/4)	0 (0/4)	
Discharge into surface waters			
Italy, Legislative Decree n. 152/2006			[34]
% of sample <5000 *E. coli*/100 mL	0 (0/4)	50 (2/4)	
Italy, Autorization Province Torino, 2012			[30]
% of sample <11,000 *E. coli*/100 mL	75 (3/4)	100 (4/4)	

Notes: [a] samples are in compliance if *E. coli* <10/100 mL in 80% of samples and <100/100 mL in the remaining samples.

To our knowledge, there are few published studies on full-scale WWTP that use PAA at low doses (1–2 mg/L) for disinfection. Zanotto et al. [28] detected ampicillin- and chloramphenicol-resistant *E. coli* in the influent and effluent from a municipal WWTP in the Milan area (Italy). The WWTP applied a final disinfection with PAA (approximately 2.0 mg/L, 45 min contact time). A reduction of *E. coli* concentration was found after the final disinfection process, resulting in >2-log units decrease in the disinfected effluent. *E. coli* at a concentration of less than 250 CFU/100 mL was observed in Finland in the effluent of a WWTP disinfected with PAA (doses of 1.5 and 2.0 mg/L; contact time 10–15 min). With these doses, a reduction of 1.68 log of *E. coli* concentration was obtained, which led to compliance with the Finnish bathing water standards (<500 CFU/100 mL) which sets the quality requirements for monitoring of public bathing waters [9]. A reduction of faecal indicators was also observed by De Luca et al. [11], who monitored the wastewaters disinfected with PAA (1.5 mg/L; contact time 18–20 min) in a WWTP located in northern Italy (equivalent inhabitants of approximately 1,000,000). The average reduction observed was 1.18 log for faecal coliforms, 1.59 log for *E. coli*, and 0.38 log for enterococci in the disinfected effluent. Zanetti et al. [14] evaluated the efficiency of PAA at low doses against some faecal indicators, analysing the effluent from a WWTP (approximately 1,000,000 equivalent inhabitants, Italy). By testing a dose of 1.2 mg/L of PAA, the following were achieved: a 1.78 log reduction of *E. coli*, a 1.23 log of faecal coliforms, and a 0.41 log for enterococci. A greater efficiency was obtained in the same study at a dose of 1.5 mg/L, which permitted a reduction of *E. coli* equal to 2.43 and 1.77 log of faecal coliforms with a contact time of 20 min; for the enterococci, a reduction of 0.66 log was observed. Comparing the results of these studies with the those of this work, they are similar if we consider the abatement achieved in spring at the higher dose of PAA (2.1 mg/L).

Monitoring *Salmonella* spp. by using the culture method revealed them to be present in all of the influent and effluent samples before the PAA treatment, whereas *Salmonella* spp. were observed in the disinfected effluent in only the winter sample (25%). In contrast, in the summer sample, *Salmonella* spp. were only detected when using the molecular method. This could be due to the different sensitivities of the two methods or to the presence of non-cultivable microorganisms [6].

In Italy, the microbiological requirements for the reuse of wastewater for irrigation are defined by the Ministry Decree of 2006 [31]; the limits prescribed for *E. coli* are <10 CFU/100 mL for 80% of the samples collected in the year and a maximum of 100 CFU/100 mL in the remaining samples. Moreover, Italian regulations include *Salmonella* spp. analysis with the culture method, requiring the total absence of the pathogen. Considering these values, although 75% of the disinfected effluents monitored in this study comply with the limit imposed for *Salmonella*, all samples exceed the value

required for *E. coli* (<10 CFU/100 mL) (Table 6). The disinfected effluents also did not comply with the microbiological standards of the WHO and the suggested value by the EPA guidelines for irrigation reuse of food crop [32,33], which allow a value of 1000 faecal coliforms/100 mL and not-detectable faecal coliforms/100 mL of wastewater, respectively.

In conclusion, the results obtained in this study highlight that although the use of low doses of PAA offers advantages in terms of cost, the production of insignificant quantities of by-products, and improvements in the microbiological quality of the effluent, these doses are not sufficient to guarantee the product's suitability for irrigation. Therefore, the need for additional studies to further assess the required dose and contact time of PAA are needed, with the aim of obtaining efficient effluent disinfection in a full-scale municipal wastewater plant.

Author Contributions: All authors made substantial contributions to the conception and design of the study and were involved in critically revising the manuscript in terms of intellectual content. In addition, Giorgio Gilli, Elisabetta Carraro and Lorenza Meucci were involved in the design study and methodology, Silvia Bonetta, Cristina Pignata and Sara Bonetta contributed to the analysis and interpretation of the data and wrote the paper, Eugenio Lorenzi and Margherita De Ceglia supported the sample collection and performed the chemical analyses. All the authors approved the final manuscript for publication.

Conflicts of Interest: The authors declare no conflict of interest.

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