Evaluation of Microbiological and Physicochemical Indicators for Wastewater Treatment

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ABSTRACT: The quality control of wastewater treatments was monitored using selected novel and classical physicochemical and microbiological indicators, and the associations of the treatments with the effluents was analyzed. The microbiological indicators monitored were heterotrophic plate count (HPC), total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), sulfite-reducing clostridia (SRC), Pseudomonas aeruginosa, and Salmonella spp. The stages of wastewater treatment also were evaluated through determination of ammonia; biological oxygen demand (BOD₅); chemical oxygen demand (COD); chloride; conductivity; suspended dissolved and total solids; fats; nitrate, nitrite, and total nitrogen; pH; phosphate and total phosphorus. Additional indicators included the Escherichia coli growth inhibition (IGEC) bioassay for assessing whole effluent toxicity, spectral determinations between wavelengths (λ) 190–650 nm, and total (TP) and soluble (SP) protein contents. Of the more common physicochemical parameters, only BOD₅, COD, suspended and total solids, and fats showed a statistically significant reduction between raw water and effluent; for the microbiological indicators, significant reduction was seen only for HPC, FC, and Ps. aeruginosa. We suggest that determinations of Ps. aeruginosa be commonly used as an indicator of wastewater quality. Spectral analysis—most notably the values of absorbance at 225, 255, and 295 nm—revealed a statistically significant correlation with several physicochemical parameters. Statistical analysis of SP and TP values showed them to be good indicators of contamination. The quantitative study of Salmonella spp. and the results of the IGEC bioassay show the need for close control of infectious and toxic risks in wastewater and effluents. © 2004 Wiley Periodicals, Inc. Environ Toxicol 19: 241–249, 2004.

Keywords: wastewater; indicators; soluble proteins; total proteins; spectral analysis; Salmonella; Pseudomonas; coliforms; fecal streptococci

INTRODUCTION

Urban wastewater contains numerous pathogenic microorganisms and a high content of organic matter; therefore, it poses a number of potential risks for public health and the environment. It was found that the serotypes of Salmonella spp. isolated from freshwater environmental sources in Spain corresponded with the serotypes found in clinical cases (Polo et al., 1999), underlining the connection between water quality and public health. Urban wastewater should be treated until an effluent free of pathogenic microorganisms and with minimal impact on the environment is
obtained. Generally, wastewater undergoes a primary treatment of decantation, then a secondary treatment that usually involves aerobic activated sludge. This conventional two-stage process is considered sufficient for eliminating pathogenic microorganisms (Gerba, 1999a). Despite the effective reduction of organic contents by such wastewater treatment, there is evidence that the effluents still contain microorganisms of great sanitary relevance, including hepatitis A virus, enterovirus, and Norwalk viruses (Gantzer et al., 1998; Espigares et al., 1999; Griffin et al., 1999).

The most frequently used microbiological indicators for detecting the different pathogens in drinking water, wastewater, and effluents are total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), sulfite-reducing clostridia (SRC), somatic coliphages, and bacteriophages of Bacteroides fragilis, each of which has a particular significance (Lucena et al., 1996; Araujo et al., 1997; Gerba, 1999b). For instance, an FC/FS ratio greater than 4 indicates fecal contamination of human origin, whereas a ratio under 0.7 would indicate contamination of animal origin (Olivieri, 1982). SRC are spore-forming and therefore more resistant, making them highly useful as indicators of past pollution and of the effectiveness of disinfection (Espigares García and Moreno Abril, 1999). The relationships between different indicators of contamination are difficult to determine in wastewater of mixed industrial and domestic composition (Espigares et al., 1996).

Because the detection of individual pathogens is expensive and laborious and may even take several days, the quality of wastewater treatments is controlled by monitoring a variety of carefully selected physicochemical indicators that reflect the type and level of contamination present in the water. Suspended solids, biological oxygen demand (BOD₅), total nitrogen, total phosphorus, fats, total solids, and solids in solution all provide information about the possible organic or fecal contamination of a water sample. Indirect chemical indicators of fecal contamination include conductivity, ammonia, phosphate, chemical oxygen demand (COD), and nitrite. Certain physicochemical parameters such as chloride and nitrate have lost their value as indicators of fecal contamination as they are increasingly present in environmental water (Espigares García and Fernández-Crehuet, 1999).

Although indicators of pollution are numerous and their assessment and interpretation the focus of much research, the development of new indicators is an additional research objective. Thus, recently there have been many studies of microbiological indicators such as sorbitol-fermenting bifidobacteria (Rhodes and Kator, 1999), bacteriophages of Bacteroides fragilis (Puig et al., 1998), somatic and F-specific coliphages (Araujo et al., 1997; Schaper and Jofre, 2000), Cryptosporidium, Giardia, and human enteric viruses (Griffin et al., 1999), and Pseudomonas aeruginosa and Aeromonas spp. (Bahlaoui et al., 1997), and of chemical indicators such as coprostanol and aminoketones (Edwards et al., 1998; Dawit et al., 2001). Also investigated have been water-quality monitoring procedures such as the fluorimetric assay of β-D-galactosidase activity (Davies and Apte, 1996), UV–visible spectrophotometric analysis (Ferree and Shannon, 2001; Muzio et al., 2001), and simple bioassays based on the inhibition of Escherichia coli growth (Espigares et al., 1990).

In the current study a series of recent and classical indicators of water quality were evaluated, and their utility in determining the quality of effluent during the wastewater treatment process was assessed. It is hoped that the breadth of this study of the microbiological composition of wastewater and effluents at various stages of treatment will improve knowledge of the effectiveness of the processes and the health and environmental risks involved.

**MATERIALS AND METHODS**

**Wastewater Samples**

Fifty-nine 1-L samples were obtained from the Granada, Spain, municipal wastewater treatment plant, which comprises two units that process the same water sources in essentially the same way and whose analytical data showed no statistically significant differences. The samples were analyzed over 7 months (October 1999—April 2000). They were collected at distinct points throughout the treatment process—from raw sewage taken at entry to the treatment plant (21 samples), from primary effluent taken after decantation (17 samples), and from treated wastewater after the activated sludge process (21 samples). The samples were integrated for 24 h with an automatic sampler. Microbiological determinations were carried out immediately on receiving the samples. When dilutions were necessary, they were made in a 0.9% saline solution and conserved at 4°C.

**Bacterial Analysis**

Several well-described classical biological indicators of contamination were chosen for inclusion in this study. TC, FC, and FS were all enumerated using the most probable number (MPN) method (APHA, 1989). In the presumptive test for coliforms, three 10-mL, three 1-mL, and three 0.1-mL volumes of the appropriate dilution of the water sample were inoculated in nine fermentation tubes with a Durham vial in MacConkey broth (BBL, Cockeysville, MD, USA). The inoculated tubes were incubated for 48 h at 37°C, and those presenting gas and acid were confirmed in Levine eosin methylene blue agar (BBL, Cockeysville, MD, USA) at 37°C for TC and in MacConkey broth with a Durham vial at 44°C for 24 h for FC.

The presumptive test for FS was performed with nine tubes of azide dextrose broth (Oxoid, Basingstoke, Hampshire, UK) to which bromothymol blue (20 mg/L) was
added as an indicator of pH. The same amounts of diluted sample were inoculated as for coliforms, and they were incubated at 37°C for 48 h. The tubes showing turbidity and acid were confirmed in ethyl violet azide broth (BBL, Cockeysville, MD, USA).

The recount of SRC spores was performed by anaerobic plate count in reinforced clostridial agar (Difco, Detroit, MI, USA) to which sodium sulfite (2 mg/L) and ammonium iron (III) citrate (1.25 mg/L) were added. The water samples were heated at 80°C for 5 min to destroy the vegetative forms.

A heterotrophic plate count (HPC) was performed using the pour plate method in standard methods agar (BBL, Cockeysville, MD, USA), incubated at 37°C for 48 h (APHA, 1989).

In addition, several other specific determinations were made. Ps. aeruginosa was quantified in a selective agar containing glycerol and cetrimide-nalidixic acid selective supplement (Oxoid, Basingstoke, Hampshire, UK) using the spread plate method, and a solution of 0.5% N,N,N′,N′-tetramethyl-1,4-phenylenediammonium dichloride (Merck) was spread to differentiate the oxidase-positive colonies. A previously described E. coli growth inhibition (IGEC) bioassay (Espigares et al., 1990, 1998) was included as a whole effluent toxicity test. The IGEC is an easy-to-perform method in which the instantaneous growth rate (IGR) of the E. coli W3110 thy− strain (CECT 416, Spanish Type Culture Collection) in sample and control cultures is compared. The IGR is calculated with the expression IGR = ln(A/ Aι) , where Aι is the absorbance at 650 nm in time t hours, and Aι−1 is the absorbance measured at time t − 1 h. Absorbances were taken for 5 h of culture in the exponential growth phase.

The enumeration of Salmonella spp. was performed using the MPN procedure (Baudart et al., 2000). Three triplicate samples of three volumes (10, 1, and 0.1 mL) were added to tubes of selective Rappaport–Vassiliadis medium (Oxoid, Basingstoke, Hampshire, UK) and incubated for 24 h at 43°C. These tubes were subsequently spread-plated onto Salmonella-Shigella agar (Oxoid, Basingstoke, Hampshire, UK) and incubated at 37°C for 48 h. Typical colonies of Salmonella spp. were transferred to triple sugar iron agar (Oxoid, Basingstoke, Hampshire, UK) and confirmed with the urease test, β-galactosidase (ONPG) test, phenylalanine test, lysine iron agar, and Kovac’s indole test. Finally, antigenic confirmation was performed by seroagglutination using commercial polyvalent Salmonella O antisera (Denka Seiken Co., Tokyo, Japan).

Physicochemical Determinations

All determinations routinely performed at wastewater treatment plants are physicochemical determinations. Those included in our study for comparison with the variables described above were: pH, conductivity, suspended solids, COD (closed reflux colorimetric method), BOD5, nitrates, nitrites, ammonia, phosphates, total nitrogen, total phosphorus, chlorides, fats, total solids, and solids in solution. All these tests were performed using standardized methodology (APHA, 1989).

Our analysis investigated several additional physicochemical parameters. Spectrophotometric measurement between the wavelengths (λ) of 190 and 650 nm was taken for each filtered sample (0.45 μm Millipore filter) using a Perkin–Elmer Lambda 2 UV–vis spectrophotometer (Überlingen, Germany). The measurement was carried out in quartz cuvettes with a path length of 1 cm, with the absorbance values noted for each wavelength (Aλ). Total (TP) and soluble (SP) protein contents were determined using the Folin reagent method (Lowry et al., 1951). The water samples were centrifuged (7000 × g, 4°C, 30 min) before soluble protein analysis; then sonified (100 W, 50% s pulses for 5 min) and centrifuged again (7000 × g, 4°C, 30 min) prior to total protein determination.

Statistical Analysis

The Student t test for two independent samples was used to assess whether there were differences in the magnitude of the indicators and in such diverse factors as stages of growth, wastewater treatment plants, and IGEC biotest. One-way ANOVA was used to compare three independent samples. In addition, a multifactorial analysis of variance was performed, controlling for the potentially confounding effect of the treatment plant. The chi-square test was used for the qualitative variables.

In evaluating the correlations between the different indicators studied, we initially calculated the simple correlation coefficients for the quantitative variables. The Student t test was applied to study the association of a positive IGEC bioassay with the rest of the quantitative indicators. Then, with each quality indicator as a dependent variable, a multiple linear regression model was constructed using the stepwise method with a p-to-enter of 0.05 and a p-to-remove of 0.10. The independent variables considered were the remaining quality parameters. For each model a corrected squared multiple correlation coefficient (cR²) was obtained, as well as the partial correlation coefficients (PCC) for each independent term in the model. The goodness of fit of the model was corroborated assessing the normality of the residual values.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows, v. 10.0).
TABLE I. Mean values and standard deviations of physicochemical and microbiological indicators at each stage of treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw Water</th>
<th>SD</th>
<th>Decanted Water</th>
<th>SD</th>
<th>Treated Water</th>
<th>SD</th>
<th>RE (%)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD₅ (mg/L)</td>
<td>317.4</td>
<td>97.6</td>
<td>274.9</td>
<td>92.6</td>
<td>75.0</td>
<td>67.1</td>
<td>76.4</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>778.4</td>
<td>210.5</td>
<td>517.5</td>
<td>82.0</td>
<td>184.2</td>
<td>142.0</td>
<td>76.6</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>962.2</td>
<td>206.6</td>
<td>926.5</td>
<td>113.9</td>
<td>919.6</td>
<td>113.5</td>
<td>4.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
<td>0.3</td>
<td>7.6</td>
<td>0.1</td>
<td>7.5</td>
<td>0.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Dissolved solids (mg/L)</td>
<td>613.5</td>
<td>259.3</td>
<td>501.7</td>
<td>180.7</td>
<td>527.1</td>
<td>261.6</td>
<td>14.1</td>
</tr>
<tr>
<td>Suspended solids (mg/L)</td>
<td>287.7</td>
<td>153.3</td>
<td>99.2</td>
<td>21.6</td>
<td>52.9</td>
<td>64.9</td>
<td>81.6</td>
</tr>
<tr>
<td>Total solids (mg/L)</td>
<td>873.9</td>
<td>282.3</td>
<td>602.1</td>
<td>182.8</td>
<td>606.1</td>
<td>235.6</td>
<td>30.6</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>53.0</td>
<td>15.4</td>
<td>59.0</td>
<td>20.1</td>
<td>51.8</td>
<td>17.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Nitrate (mg/L)</td>
<td>6.0</td>
<td>5.0</td>
<td>4.9</td>
<td>3.7</td>
<td>6.2</td>
<td>5.3</td>
<td>-3.3</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.4</td>
<td>0.4</td>
<td>1.9</td>
<td>1.9</td>
<td>-850</td>
</tr>
<tr>
<td>Total nitrogen (mg/L)</td>
<td>185.8</td>
<td>107.7</td>
<td>166.9</td>
<td>91.6</td>
<td>130.1</td>
<td>48.4</td>
<td>30.0</td>
</tr>
<tr>
<td>Chloride (mg/L)</td>
<td>80.4</td>
<td>28.7</td>
<td>77.5</td>
<td>25.9</td>
<td>73.9</td>
<td>27.4</td>
<td>8.1</td>
</tr>
<tr>
<td>Phosphate (mg/L)</td>
<td>33.8</td>
<td>10.7</td>
<td>33.8</td>
<td>11.3</td>
<td>28.2</td>
<td>9.8</td>
<td>16.6</td>
</tr>
<tr>
<td>Total phosphorus (mg/L)</td>
<td>45.6</td>
<td>30.6</td>
<td>41.7</td>
<td>34.6</td>
<td>33.7</td>
<td>23.6</td>
<td>26.1</td>
</tr>
<tr>
<td>Fats (mg/L)</td>
<td>105.3</td>
<td>59.1</td>
<td>78.3</td>
<td>54.6</td>
<td>32.5</td>
<td>36.1</td>
<td>69.1</td>
</tr>
<tr>
<td>Soluble proteins (mg/L)</td>
<td>60.1</td>
<td>21.1</td>
<td>50.5</td>
<td>11.6</td>
<td>24.1</td>
<td>11.1</td>
<td>59.9</td>
</tr>
<tr>
<td>Total proteins (mg/L)</td>
<td>112.5</td>
<td>31.2</td>
<td>72.6</td>
<td>15.0</td>
<td>41.0</td>
<td>19.6</td>
<td>63.6</td>
</tr>
<tr>
<td>HPC (cfu/mL)</td>
<td>1.3 × 10⁶</td>
<td>1.1 × 10⁶</td>
<td>9.0 × 10⁶</td>
<td>0.9 × 10⁶</td>
<td>0.4 × 10⁶</td>
<td>0.5 × 10⁶</td>
<td>69.2</td>
</tr>
<tr>
<td>TC (MPN/100 mL)</td>
<td>1.1 × 10⁸</td>
<td>2.4 × 10⁸</td>
<td>8.0 × 10⁸</td>
<td>2.2 × 10⁸</td>
<td>0.2 × 10⁸</td>
<td>0.3 × 10⁸</td>
<td>81.8</td>
</tr>
<tr>
<td>FC (MPN/100 mL)</td>
<td>8.2 × 10⁶</td>
<td>4.2 × 10⁶</td>
<td>6.9 × 10⁶</td>
<td>9.7 × 10⁶</td>
<td>1.9 × 10⁶</td>
<td>2.3 × 10⁶</td>
<td>76.8</td>
</tr>
<tr>
<td>FS (MPN/100 mL)</td>
<td>8.2 × 10⁶</td>
<td>2.0 × 10⁶</td>
<td>3.8 × 10⁶</td>
<td>5.0 × 10⁶</td>
<td>0.4 × 10⁶</td>
<td>0.3 × 10⁶</td>
<td>95.1</td>
</tr>
<tr>
<td>SRC (cfu/20 mL)</td>
<td>1.5 × 10⁵</td>
<td>4.0 × 10⁵</td>
<td>1.3 × 10⁵</td>
<td>3.8 × 10⁵</td>
<td>0.2 × 10⁵</td>
<td>0.1 × 10⁵</td>
<td>86.7</td>
</tr>
<tr>
<td>Ps. aeruginosa (cfu/mL)</td>
<td>3872.2</td>
<td>2854.9</td>
<td>4242.9</td>
<td>2691.3</td>
<td>595.6</td>
<td>970.7</td>
<td>84.6</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>266.7</td>
<td>475.4</td>
<td>81.0</td>
<td>150.6</td>
<td>45.0</td>
<td>120.6</td>
<td>83.1</td>
</tr>
</tbody>
</table>

¹ Each value is for both treatment plants combined where no significant difference between stations was found.
² Statistical significance of differences between raw and treated water (independent-sample t test, p < 0.05).

RESULTS AND DISCUSSION

Parameters

Mean values were calculated for all parameters at each stage of treatment (Table I). Of the physicochemical parameters, only BOD₅, COD, fats, suspended solids, and total solids showed statistically significant reduction during treatment; in fact, the mean values of BOD₅ and COD decreased to 76.4% and 76.6%, respectively. Of the suspended solids, 65.5% were eliminated with primary decantation, and 81.6% were eliminated in the final effluent. Meanwhile, the ammonia and nitrate content was not significantly reduced through treatment, and nitrites underwent a noteworthy increase, from 0.2 mg/L in raw water to 1.9 mg/L in treated water. These results confirm the value of BOD₅, COD, and suspended solids in the control of wastewater treatment. Conductivity, although simple to determine and very useful for the quality control of drinking water, is inadequate for monitoring sewage. This was confirmed by the linear regression of conductivity with COD (r = 0.162, p = 0.221), BOD₅ (r = 0.007, p = 0.961), and suspended solids (r = 0.075, p = 0.573), findings partially described by Muzio et al. (2001), as they found no relationship between conductivity and BOD₅.

Soluble and total proteins showed significant differences (p < 0.05) between raw and treated water. During primary decantation 16% of the soluble proteins were eliminated, as were 35.5% of total proteins. The activated sludge process removed 53.3% of the soluble proteins and 43.5% of the total proteins. These data indicate that soluble proteins constitute a quantitatively better indicator than do total proteins for the assessment of the biological stage of wastewater treatment.

The microbiological removal efficiency rates of our study resembled those of other published data (Bahlaoui et al., 1997; Saleem et al., 2000). Of the microbiological parameters most habitually determined, only HPC and FC showed significant differences (p < 0.05) between raw and treated water. As shown in Table I, the SRC content in was recorded by Chauret et al. (1999) during an analogous wastewater treatment process; yet our observed decreases in HPC, TC, FC, and FS were inferior to those of the aforementioned authors.
Ps. aeruginosa showed a statistically significant reduction that was of the same order as magnitude as the rest of the microbiological parameters (Table I) but did not exhibit a relationship with proteins (Table VI) or with Salmonella (Table VII). Although Pseudomonas is an environmental microorganism and opportunistic pathogen, it is acquiring greater importance as an indicator in treatment processes and in the control of the quality of wastewater, drinking water, swimming pool water, and recreational waters. This microorganism has been reported to appear in feces, most likely human rather than animal (Olivieri, 1982). Bahgat et al. (1999), who studied the buildup and distribution of microorganisms in sand filters used for wastewater treatment, identified Ps. cepacia in the upper layer of the filter, and Ps. aeruginosa and Ps. fluorescens in the bottom layer of the filter, thereby showing members of the genus Pseudomonas to be commonly present in the biological treatment of wastewater. Veschetti et al. (2003) included Pseudomonas sp. in a study of the disinfectant capacity of peracetic acid and sodium hypochlorite in a wastewater pilot plant. Maurines-Carbonell et al. (1998) also used Pseudomonas as a parameter in their study of the control of wastewater treatment plant dysfunction in cases of underaeration. All the above findings, among others, were supported by the results of the current study, in that Ps. aeruginosa was significantly reduced during treatment, which had no statistically significant correlation with other parameters. For this reason, it may be a particularly useful indicator in evaluating wastewater treatment.

**IGEC Bioassay**

This bioassay has proven useful for the control of toxicity in water and aqueous solutions (Espigares et al., 1990, 1998). In the present study, 31 samples were analyzed with the IGEC bioassay, of which 10 were positive (32.3%). The distribution of the positive samples in each stage of treatment is shown in Figure 1. The percentage of positive results decreased progressively over treatment: 50 for raw, 25 for decanted, and 18.2 for treated water, though the chi square test showed that these differences were not significant ($p = 0.23$).

**Fig. 1.** Percentage of samples with a positive IGEC bioassay in each stage of treatment ($\chi^2 = 2.919; p = 0.232$).

**Spectral Analysis**

Figure 2 shows the spectral analyses of raw and treated waters. The absorbance values were significantly different.

**TABLE II.** Parameters presenting a statistically significant Pearson correlation with absorbance values at different wavelengths (interval $\lambda_{225}$–$\lambda_{505}$); $\lambda$ values shown present a maximum correlation coefficient for some parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rangea</th>
<th>$\lambda$ (nm)</th>
<th>$r^b$</th>
<th>$p^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD$_5$</td>
<td>235–500</td>
<td>255</td>
<td>0.423</td>
<td>0.010</td>
</tr>
<tr>
<td>COD</td>
<td>220–505</td>
<td>295</td>
<td>0.492</td>
<td>0.002</td>
</tr>
<tr>
<td>Conductivity</td>
<td>225–335</td>
<td>225</td>
<td>0.414</td>
<td>0.000</td>
</tr>
<tr>
<td>Phosphate</td>
<td>255–505</td>
<td>300</td>
<td>0.401</td>
<td>0.015</td>
</tr>
<tr>
<td>Soluble proteins</td>
<td>225–505</td>
<td>255</td>
<td>0.693</td>
<td>0.000</td>
</tr>
<tr>
<td>Total nitrogena</td>
<td>260–435</td>
<td>340</td>
<td>0.404</td>
<td>0.022</td>
</tr>
<tr>
<td>Total proteins</td>
<td>230–325</td>
<td>245</td>
<td>0.451</td>
<td>0.005</td>
</tr>
</tbody>
</table>

a Interval of wavelength in which the correlation with the absorbance values is significant.
b Pearson correlation coefficient.
c Level of statistical significance.

Though presenting significant correlations in the range 260–435 nm, the correlation is not significant for absorbance values 285 through 305.
in the raw and treated water samples only in the \(\lambda\) interval of 225–505 nm. The greatest differences between raw and treated water in the mean values of absorbance were seen at \(\lambda = 225\) and \(\lambda = 230\), a difference of 0.44 between the two \(\lambda\) values. Although spectral analysis is rarely applied to wastewater assessment, authors Muzio et al. (2001) registered UV absorbance to estimate the BOD\(_5\) of effluents from two pulp-and-paper mills that used different processes. Interestingly, the effluent from the pulp mill investigated in their study showed a spectrum very similar to that in our study, as shown in Figure 2.

Of all the physicochemical parameters determined, only the ones shown in Table II had a statistically significant correlation with the absorbance values for some wavelengths. The range of wavelengths for each parameter with significant correlation was very wide, that is, there was no specificity of the wavelengths for a given parameter. However, the maximum correlation coefficient of Pearson was obtained for soluble proteins at \(\lambda = 255\) nm (Table II). To analyze the form in which the distinct parameters could affect absorbance in the aforementioned wavelengths (Table II), stepwise multiple linear regression models were con-
structed. In Table III we include as possible independent variables all the parameters except the microbiological ones, fats, pH, suspended solids, and total solids, which are not directly related to absorbance. Nor are soluble and total proteins included, so that the behavior of the more commonly determined chemical indicators becomes evident. COD is the parameter best related to absorbance, followed by dissolved solids in absorbances at 295 and 300 nm. For absorbance at 225 nm, aside from conductivity, no new variable appears in the model. These results show that the UV absorbance values considered in Table III are good predictors of COD. Furthermore, A295 and A300 could be used as a combined indicator of COD and dissolved solids.

Soluble and Total Proteins

Although the variables total and soluble proteins appear in the stepwise linear regression models (Table IV), the variable that showed the strongest association is soluble proteins—at A275 a maximum $cR^2$ value of 0.460 was seen. Only above 295 nm did a second variable appear in the model—dissolved solids. These data are consistent with the close association between the variables COD, soluble proteins, and absorbance at different wavelengths in the ultraviolet range, most notably at A255. The strong relationship between soluble proteins and COD was confirmed in the linear regression models shown in Table V.

Another important aspect of soluble and total proteins is their potential as indirect indicators of fecal contamination. As can be seen in Table VI, both types of proteins had a significant correlation with HPC, showing them to be indicators of total bacterial load. At the same time, however, an important distinction between soluble and total proteins was shown. The stepwise linear regression model with soluble proteins as a dependent variable introduced SRC, a long-lived microbiological parameter, as the second variable. Yet when total proteins were taken as a dependent variable, TC and FS appeared in the model, both of which are more specifically related to short-term pollution (Olivieri, 1982; Edberg et al., 1997).

Relationships Between Salmonella and Indicators

The raw water samples show a high content of Salmonella spp., with a mean MPN of 266.7/100 mL (Table I). This finding should be underlined as a matter of concern, because it was accurately reflected in a high number of isolations of Salmonella spp. in clinical samples from Andalusian hospitals, as documented by Spain’s Sistema de Información Microbiológica de Andalucía (SIMAN, 2001). In theory, pathogenic bacteria undergoing wastewater treatment would be eliminated by sedimentation and the biological processes of competitive elimination. In reality, this is not so: the

### TABLE V. Stepwise linear regression models for total proteins and soluble proteins with the physicochemical parameters

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variables</th>
<th>Step</th>
<th>Coefficient</th>
<th>$p$</th>
<th>PCC</th>
<th>$cR^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble proteins</td>
<td>COD</td>
<td>1</td>
<td>$5.28 \times 10^{-2}$</td>
<td>0.000</td>
<td>0.701</td>
<td>0.417</td>
</tr>
<tr>
<td></td>
<td>Total phosphorus</td>
<td>2</td>
<td>$-0.182$</td>
<td>0.034</td>
<td>$-0.241$</td>
<td>0.462</td>
</tr>
<tr>
<td>Total proteins</td>
<td>COD</td>
<td>1</td>
<td>$0.108$</td>
<td>0.000</td>
<td>0.839</td>
<td>0.619</td>
</tr>
<tr>
<td></td>
<td>Total phosphorus</td>
<td>2</td>
<td>$-0.315$</td>
<td>0.007</td>
<td>$-0.245$</td>
<td>0.671</td>
</tr>
</tbody>
</table>

* Parameters included: conductivity, COD, BOD$_5$, ammonia, nitrite, nitrate, phosphate, total nitrogen, total phosphorus, chloride, and dissolved solids.

* Step in which each independent variable was introduced.

* The coefficients, $p$ value, and PPC (partial correlation coefficient) displayed correspond to those of the final model (in the last step).

* $cR^2$: Multiple correlation coefficient squared obtained in each step.

### TABLE VI. Stepwise linear regression models for total and soluble proteins with the microbiological parameters

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variables</th>
<th>Step</th>
<th>Coefficient</th>
<th>$p$</th>
<th>PCC</th>
<th>$cR^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble proteins</td>
<td>HPC</td>
<td>1</td>
<td>$2.57 \times 10^{-5}$</td>
<td>0.001</td>
<td>0.598</td>
<td>0.328</td>
</tr>
<tr>
<td></td>
<td>SRC</td>
<td>2</td>
<td>$1.68 \times 10^{-5}$</td>
<td>0.048</td>
<td>0.315</td>
<td>0.407</td>
</tr>
<tr>
<td>Total proteins</td>
<td>HPC</td>
<td>1</td>
<td>$6.72 \times 10^{-5}$</td>
<td>0.000</td>
<td>0.812</td>
<td>0.447</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>2</td>
<td>$6.42 \times 10^{-7}$</td>
<td>0.001</td>
<td>0.465</td>
<td>0.613</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>3</td>
<td>$-3.83 \times 10^{-6}$</td>
<td>0.040</td>
<td>$-0.361$</td>
<td>0.663</td>
</tr>
</tbody>
</table>

* Parameters included: HPC, TC, FC, SRC, Pseudomonas, and Salmonella.

* Step in which each independent variable was introduced.

* The coefficients, $p$ value, and PPC (partial correlation coefficient) displayed correspond to those of the final model (in the last step).

* $cR^2$: Multiple correlation coefficient squared obtained in each step.
treated water contained a Salmonella MPN of 45/100 mL, representing a reduction of only 83%. Thus, the effluent stands as a public health risk in the transmission of salmonellosis, as already confirmed by other authors (Kinde et al., 1997).

Stepwise linear regression models were constructed separately for the physicochemical and the microbiological parameters (Table VII) in order to determine the best predictors of Salmonella spp. content. This, in turn, allowed us to establish the best indicators of this pathogenic microorganism at each of the stages of wastewater treatment considered. Of the physicochemical variables investigated, COD showed the strongest association with Salmonella, whereas TC was the best indicator among the microbiological variables. It is very noteworthy that FC and FS, traditionally considered to be highly specific indicators of fecal contamination (Edberg et al., 1997), did not appear in our model. There was no statistically significant correlation between Ps. aeruginosa and the other parameters, leading us to question its accuracy as an indicator of fecal contamination, although the reduction during the treatment per se was statistically significant. Thus, it may prove to be more appropriate for monitoring wastewater treatment.

### CONCLUSIONS

Our analysis of wastewater subjected to a conventional treatment of primary decantation and activated sludge process suggests that not all the familiar physicochemical parameters are accurate indicators of effluent quality. In fact, only BOD₅, COD, suspended and total solids, and fats showed a statistically significant reduction between raw water and effluent, thus confirming the value of these indicators for the control of treatment. The microbiological indicators did not show an important reduction over treatment. Statistically significant decreases were limited to HPC, FC, and Ps. aeruginosa, pointing to a greater usefulness of these indicators, and leading us to suggest the determinations of Ps. aeruginosa, infrequently used to date as a microbiological indicator for water treatment assessment.

The absorbance values obtained in the spectral analysis showed a wide range of wavelengths bore statistically significant correlations with several physicochemical parameters. Potential control parameters are: absorbance at 225 nm, especially related to conductivity; absorbance at 255 nm, which showed a greater correlation with COD; and absorbance at 295 nm, which might be used as an combined indicator of COD and dissolved solids.

The values obtained in the determination of soluble and total proteins and the statistical analysis of these proteins make manifest their potential as indicators of contamination in the control of wastewater treatments. The values obtained in the spectral analysis by linear regression showed that soluble proteins had a maximum correlation coefficient with absorbance at 275 nm and a good correlation with absorbance at 255 nm. This would indicate the great utility of soluble proteins as a commonly used indicator, especially in light of the simplicity of its determination.

From the results of the quantitative study of Salmonella spp., COD and TC stand out as the best indicators of these pathogenic species, leading us to conclude that the conventional treatment of wastewater with primary decantation and an aerobic biological treatment is insufficient for disinfecting wastewater.

Finally, a simple bioassay based on the inhibition of the growth of E. coli had positive results in a considerable percentage of the samples, indicating the utility of this bioassay for controlling toxicity in wastewater and effluents.

### REFERENCES


