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STUDY OF TOXICITY ASSOCIATED TO DUMPING OF WASTEWATER CONTAINING DYES AND PIGMENTS IN THE ABURRÁ VALLEY METROPOLITAN AREA

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ABSTRACT

Dyes and pigments are beginning to be considered in this country as compounds that can present toxicological characteristics beyond esthetic aspects of wastewater. This paper presents the ecotoxicological effects associated with the capacity to inhibit light transmission in an aquatic medium with color generating substances (dyes and pigments), as well as the toxicity associated the molecule that makes it up. From an experimental design a correlation was made of the effect of the concentration, total content of solids and maximum absorption wavelength of of different dyes in the *Selenastrum Capricornutum* organism. The regression models obtained enabled predicting, in 94 and 99%, the aforementioned effects. Molecular toxicity was evaluated using the *Daphnia magna* toxicity test. Lastly, the toxicity of wastewater dumping corresponding to the leading industrial sectors of the Metropolitan Area of the Aburrá Valley.

KEYWORDS: Dyes, pigments; Wastewater, toxicity tests; *Daphnia magna*; *Selenastrum capricornutum*; Analysis of variance (ANOVA).

ESTUDIO DE LA TOXICIDAD ASOCIADA AL VERTIMIENTO DE AGUAS RESIDUALES CON PRESENCIA DE COLORANTES Y PIGMENTOS EN EL ÁREA METROPOLITANA DEL VALLE DE ABURRÁ

RESUMEN

Los colorantes y pigmentos están comenzando a ser considerados en el país como compuestos que pueden presentar características toxicológicas más allá de los aspectos estéticos en las aguas residuales. Esta investigación presenta los efectos ecotoxicológicos asociados a la capacidad que poseen las sustancias generadoras de color (colorantes y pigmentos) de inhibir la transmisión de la luz en el medio acuático, además de la toxicidad asociada a la molécula que las constituye. A partir

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de un diseño experimental se correlacionó el efecto de la concentración, contenido de sólidos totales y la longitud de onda de máxima absorción de diferentes colorantes sobre el organismo *Selenastrum Capricornutum*; los modelos de regresión obtenidos permitieron predecir en un 94 y 99% los efectos anteriormente mencionados. La toxicidad molecular fue evaluada utilizando la prueba de toxicidad de *Daphnia magna*. Finalmente se estudió la toxicidad de vertimientos de aguas residuales correspondientes a los principales sectores industriales del Área Metropolitana del Valle de Aburrá.

PALABRAS CLAVE: Colorantes; pigmentos; aguas residuales; pruebas de toxicidad; *Daphnia magna*; *Selenastrum capricornutum*; Análisis de varianza (ANOVA).

ESTUDO DA TOXICIDADE ASSOCIADA AO VERTIMENTO DE ÁGUAS RESIDUAIS COM PRESENÇA DE CORANTES E PIGMENTOS NA ÁREA METROPOLITANA DO VALLE DEL ABURRÁ

RESUMO

Os corantes e pigmentos estão começando a ser considerado no país como compostos que possam ter características toxi- cológicas além dos aspectos estéticos nas águas residuais. Esta investigação apresenta os efeitos ecotoxicológicos associadas à capacidade que possuem as substâncias geradoras de cor (corantes e pigmentos), para inibir a transmissão de luz no meio aquático, além da toxicidade associada à molécula que as constitui. A partir de um desenho experimental se correlacionou o efeito da concentração, conteúdo de sólidos totais e o comprimento de onda de absorção máxima de diferentes corantes sobre o organismo *Selenastrum capricornutum*; modelos de regressão obtidos permitiram predizer em um 94 e 99% os efeitos anteriormente mencionados. A toxicidade Molecular foi avaliada usando o teste de toxicidade de *Daphnia magna*. Finalmente, estudo-se toxicidade de efluentes de águas residuais correspondentes aos principais sectores industriais da Região Metropolitana do Valle de Aburrá.

PALAVRAS-CHAVE: Corantes; Pigmentos; Águas residuais; Testes de toxicidade; *Daphnia Magna*; *Selenastrum capri-Cornutum*; Análise de variância (ANOVA).

1. INTRODUCTION

Currently, the contamination of aquatic systems is a universal phenomenon that greatly affects developing countries as a consequence of the dumping of untreated or partially treated wastewater. Among the greatest concerns that wastewater dumping causes on hydric sources, the fact of their toxicity is highlighted, along with the negative esthetic impacts that frequently occur because of the dumping of dyes and pigments, that affect, among other aquatic organisms, those with the capacity for photosynthesis processes, which constitute the base of primary producers that guarantee energy flows at higher trophic levels (Sharma, 2007; Rand, 1995; Bae & Feeman, 2005; Verma, 2011).

At a local level, communities belonging to the Aburrá Valley Metropolitan Area, have experienced constant wastewater discharge from some manufacturing sectors, which produce changes in the base color of the Aburrá-Medellín River. This motivated the development of different studies that enable advancement in determining real effects that dyes and pigments cause on the aquatic receptor ecosystems. Consequently, the present research is conducted aiming to find out and analyze the different types of toxicity associated to wastewater dumping containing dyes and pigments on hydric sources. Results obtained also enabled defining the grade of toxicity of wastewater containing dyes, the type of the most optimal toxicity test or protocol to evaluate these, considering standing Colombian regulation (Resolution 062 of 2007) effluents.

The classification of pigments and dyes can be conducted using the following as criteria: molecular constitution or chemical structure, grade of solubility in a medium or specific solvent and the form of application on an object fiber or support material. The latter classification is adopted by the Color Index system, which contains a five-digit number corresponding to the respective name and color. The Color Index is comprised of the following dye and pigment groups: acid, basic, disperse, direct, reactive and vat (Gupta, 2009).

The aforementioned classification was considered in the study of ecotoxicity of wastewater with a presence of dyes and pigments in the Metropolitan Area of the Aburrá Valley. Nine manufacturing sectors were selected which were related to the use of raw goods and wastewater dumping containing dyes and pigments in the Aburrá – Medellín River. These sectors included textile, chemical, tannery, graphic arts, metal-mechanics, food and drink. Participating companies were initially selected according to the ranking of the 500 largest in Antioquia, per RAED magazine published by the Medellín Chamber of Commerce (2011). They were so defined according to the participating index of each one of the manufacturing sectors in reported net sales. It was commonly accepted that the higher the sales, the higher the increase in manufacturing, and therefore in raw goods used and, in turn, the generation of wastewater. From reports of raw goods used by manufacturers and taking into account the characterization of wastewater dumped by companies, ten (10) of the most used substances (between dyes and pigments) in manufacturing processes were selected. This was done in order to cover the total visible spectrum in terms of wavelengths and maximum absorbencies, including white and black tones. Taking all this into account, the ecotoxicity of the colorants was analyzed: Crimson Indigo, Red 40, Yellow N° 5, Basic Chrome Sulphate, Black Iron Oxide, Titanium Dioxide, Zinc Oxide, Rhodamine B, Copper Oxide, Red Iron Oxide.

The aquatic ecotoxicity is the qualitative and quantitative study of the adverse or toxic effects chemical substances, as well as other xenobiotic materials, produce at different organization levels, from cell level, through individual organisms, to communities and ecosystems in general. Toxicity includes lethal short and long term effects, additionally considering sub-lethal effects, represented by changes in growth, development, reproduction and behavior, as such, a relative property of the substance´s potential chemistry to which live organisms are exposed, depending on the concentration, composition, chemical properties of substances and the exposure time of the microorganisms.

Toxicity tests are used to evaluate the adverse effects of a chemical component on live organisms under standard and reproductive conditions, which enables comparison to other chemical compounds or evaluated species. The acute toxic effects are determined in short times of exposure, relative to the exposed organism's life cycle. They are generally severe or deathly and tend to be expressed in terms of average lethal concentration (CL50), value which defines the concentration to which the number of organisms used in the toxicity test is reduced to 50% during a period between 96 hours and 14 days. The chronic toxic effect, on the other hand, is associated to frequent exposure to a specific substance or group of substances during long periods of time. During these periods, there may be phases of latency which make the observation of toxic effects difficult. These phases depend on the type of organisms, the characteristics of the applied toxicity test and the chemical properties of the substances involved. As a consequence, they may produce lethal effects (Rand, 1995).

Different techniques exist to evaluate wastewater toxicity. These generally use organisms of various trophic levels, among which the following stand out: bacteria, protozoa, crustaceans, and even higher organisms such as frogs, fishes, rats, mice, human cells, etc.

The bioassays provide valuable information with respect to the toxicity of mixed solutions on live organisms. The *Daphnia magna*, is a micro crustacean that has been widely used as a test organism in acute and chronic toxicity studies of a variety of chemical compounds present in aquatic ecosystems. *D. magna*, is the most used species in the world because of its sensibility to toxic agents. These micro crustaceans have a short cycle and reproduce by parthenogenesis, being easy to manipulate in the lab (United States Environmental Protection Agency, EPA, 2002). *Vibrio fischeri,* is a luminescent marine bacterium that rapidly and with high sensitivity can detect a variety of toxic compounds. Toxic chemical products inhibit the electron transference process of the transport chain of *V. fischeri* reducing the capacity of light production. This bioassay can be used as a toxicity indicator in mammals, due to the existing gene similarity and compatibility between these organisms and *V. fischeri* (Wang, 2010; Rosenkranz, 1993).

Micro algae make up the first link in the trophic chain of aquatic systems. In its condition as primary producer, phytoplankton enable the entrance of energy to the ecosystem, regulates gaseous system and has a purifying effect, which is why it is considered one of the most important indicators of alterations in marine medium. Its sensibility to industrial effluents has been verified and it has demonstrated being a valid tool in the monitoring and control of toxicity reduction programs. It is also considered a leading indicator of contamination (Bafana, 2011). The microalgae *Selenastrum capricornutum* is sensitive to toxic substances and, as such, is used in aquatic toxicity tests.

The state of the art presented below shows results found after the use of techniques for toxicity measurement in aquatic media associated discharge of dyes and pigments. In addition to the preceding, chapter two (2) presents the current regulatory framework for toxicity tests in Colombia.

2. TECHNIQUES FOR ACUTE TOXICITY ANALYSIS IN WATERS ENDORSED BY COLOMBIAN LEGISLATION

Under Colombian regulation, Resolution 0062 of 2007 defines laboratory protocols required for the development of environmental analysis, among them those concerning acute toxicity evaluation in waters. The Resolution contemplates, as main protocols and methods for the determination of water toxicity, the *Daphnia magna* immobilization test, inhibition of algae growth and the bacteria bio-luminescence test (with *Vibrio fischeri* bacteria). Also, in those cases in which the result of acute toxicity at *D. magna* algae inhibition is close to the 50% limit, it establishes there must be an additional evaluation conducted with fish.

3. STATE OF THE ART AQUATIC TOXICITY TESTS

3.1 Acute toxicity for Daphnia

Among the toxicity studies applied on Daphnias, we evaluated the effect of direct blue dye 218, which presented a high toxicity with values of LC_{50} between 3,6 and 6,0 mg/L. According to Bae and Freeman, the adverse effects generated in *Daphnias* are enough to predict possible damages and impacts on all ecosystems with wastewater dumping contaminated with the substance used in the analysis. This proves the need to conduct toxicological studies in industries related to chemical products, among them, synthetic dye industries (Bae y Freeman, 2005). In India, Verma (2008) applied the acute toxicity technique *Daphnia magna* on industrial effluents containing reactive dyes Remazol *green parrot* and Remazol yellow, obtaining effective concentration values for a percentage of inhibition of 50% of the total population of *Daphnias* (EC_{50}), from 55,32 and 46,84 mg/L respectively. The author indicates that the trial with *Daphnia magna*, is an excellent method to evaluate the aquatic toxicity of stains and dyes that contain industrial effluents. In general, the effluents with a presence of dyes and pigments belonging to the tannery sector showed a greater amount with respect to dumping from sectors such as textiles and paper (Verma, 2011). Dave and Aspegren used *Daphnia magna* to determine the toxicity of textile effluents according to the protocol established in the ISO Standard 6341 of 1982. For this, 52 samples of textile wastewater dumping were taken. The values of EC_{50} obtained were defined within a range of 3,7 and 118 g/L after 24 hours of exposure and within 1 and 182 g/L after 48 hours of exposure. To demonstrate the provided toxicity for textile effluents, Immich carried out an evaluation of toxicity of solutions of the reactive dye Remazol blue. All the analyzed samples showed acute toxicity above the maximum limit allowed for effluents from the textile industry and chemical products (Immich, 2009).

2.2 Inhibition of algae growth

With the aim of studying the size of particulates and the solubility of metal oxidants, Aruoja and Dubourguiera (2009) compared the toxicity of nanoparticles of ZnO, TiO_2 and CuO in its regular size. For the bioassays, *Pseudokirchneriella subcapitata* algae was used. In spite of being opaque, aqueous solutions did not show a significant effect on growth inhibition of algae with respect to the capacity these substances have to reduce the passing of light and affect photosynthesis processes. Both the large-sized Zinc Oxide particles and the nanometer presented similar EC_{50} toxicity values of 0,037 and 0,042 mg/L (for 72 hours of exposure), respectively. This proves a high grade of toxicity attributed to dissolved Zinc ions. The results were coherent with those reported by Franklin, Rogers and Apte (2007), who, when comparing normal sized molecules to their nanoparticle, using the same organism (*Pseudokirchneriella subcapitata*), obtained EC_{50} values during 72 hours of exposure 0,068 and 0,063 mg/L, for the nanoparticles and the normal molecule. In the case of the Titanium Dioxide, the reported toxicity was considerably less than the other metal oxides. EC_{50} values were determined of 5,8 and 35,9 mg Ti/L for 72 hours of exposure for the nanoparticle and the normal molecule. Both solutions formed aggregates that trapped microorganisms impeding passage of light and thus inhibiting its growth. These values indicate that the greatest degree of toxicity is shown by nanoparticles of TiO_2. One possible explanation for this effect can be the one suggested by Baveye and Laba (2008) who focused their research on the toxicological implications of the aggregates of the nanoparticles of Titanium Dioxide, due to the fact they can result in different biological activities. The authors explained the toxicity mechanisms of suspensions, as in the case of the generation of hydroxyl radicals because of visible light. The TiO_2 nanoparticles combined with ultraviolet light (370 nm) inactivate the algae. Something similar happened with the Copper Oxide where the nanoparticles resulted more toxic than the normal-sized molecules, presenting EC_{50} values of 71 and 11,55 mg/L during 72 hours of exposure for nanoparticles and normal molecules, respectively. The toxicity of ZnO and CuO can be attributed to soluble metal ions. In the case of the TiO_2, the entrapment of the algae on the TiO_2 aggregates can play an important role in toxicity on *Pseudokirchneriella subcapitata* (Aruoja and Dubourguiera, 2009).

Sponza (2006) researched acute toxicity of wastewater of the chemical dye industry and highlights the importance of toxicity tests in the regulations for wastewater dumping. He used different organisms to represent four trophic levels, including bacteria, protozoa, fishes and *Chlorella vulgaris algae*. The wastewater contained several types of dyes, where indigo blue was present in the greatest proportion. The acute toxicity tests for these effluents showed values of EC_{50} (expressed as a percentage of the effluent sample studied) between 80 (that is, taking 80% wastewater sample and 20% distilled water) and 100% (in which 100% of wastewater is taken, and 0% distilled water) indicating low levels of toxicity. Generally, results of acute toxicity tests are expressed in EC_{50}, which means this is the concentration that affects 50% of the trial organisms in different effluent volumes. Novotny (2006) conducted a study on some reactive and disperse dyes to compare the use of three different toxicity tests using, as organisms, *Vibrio fischeri* bacteria, *Selenastrum capricornutum* microalgae and *Tetrahymena pyriformis* protozoa. Although the bioluminescence test determines toxicity, it has low sensitivity to dark dyes and needs more application time. The algae growth inhibition test was more sensitive to dyes in comparison to the bioluminescence test. *T. pyriformis* is an adequate organism to measure biological toxicity, although not as sensitive as algae. Other comparative studies have been conducted focused on the toxicity of several dyes, including acid, reactive, direct and vat types, which are commonly found in textile wastewater. Tigini (2011) compared the techniques with *Daphnia magna*, *Vibrio fischeri, Pseudokirchneriella subcapitata*, the Ames test with Salmonella typhimurium and the Lemna test with Lemna minor. It was concluded from the results, that *D. magna* is the organism that is most sensitive to effluents with a presence of acid dyes, which have a formation of foam. This way, surfactants that contain these dyes can seriously affect the Daphnias. As such, *D. magna*, is probably useful o evaluate the effect of the "physical toxicity" caused by surfactants undetectable by other organisms. The bioluminescence test with *V. fischeri* bacteria showed limitations during the evaluation of toxicity of pigments (insoluble substances) and vat dyes among others that produce dark colors. The research determined these limitations are produced by color interference and suspended material (pigments) on light emission of the bacteria. The Pseudokirchneriella subcapitata algae was the most sensitive organism for the majority of wastewater tested, which suggests its use in studies about the ecotoxicology of effluents of the textile and tannery sectors.

4. MATERIALS AND METHODS

4.1. Prediction models for toxicity associated to wastewater discharge with the presence of dyes and suspended solids

For the purpose of generating a statistical model that enables predicting the adverse effects produced on aquatic organisms associated to the capacity dyes and suspended solids possess for inhibiting the transmission of light in the water and, consequently, reducing the photosynthesis processes, two experiment designs were developed type Central Compound, using the statistic software DESIGN EXPERT ® V.9 (Stat-Ease, 2014), which has the capacity to establish mathematics or polynomial models of correlation between the main variables and the response variable with a high precision grade and statistical significance represented by the variance analysis (ANOVA). In both designs, a response variable that was used was the toxicity in algae determined from the algae growth inhibition test (ISO Standard 8682). The selection of the toxicity test was conducted taking into account results and recommendations quoted by different authors, as well as the reduced limitations the test presents when dyes are used. This, in contrast to the bioluminescence bacteria test in which dyes and suspended solids interfere with measurement.

The statistical models developed to estimate toxicity associated to a concentration of a specific dye and total solids present in wastewater, will enable in the future, once calibrated and validated, making predictions and quantifications of the adverse effects generated on aquatic organisms, from the physical characterization of the effluent in terms of color and total solids.

4.1.1 Inhibition of algae growth associated to dyes

Design of experiments

For the evaluation of toxicity associated to dyes in water, according to the experimental design, five vegetable anilines were used, that were frequent in the food industry, which reduce the adverse effects associated to molecule ingredient of the dye and show the effects related to the decrease in light transmission in the aquatic media.

The experimental design of the Central Compound used, as a response variable, the toxicity expressed in percentage which represents the reduction of the cell duplication rate of the *Selenastrum Capricornutum* organism. As main variables (those which have direct influence of the phenomenon or process), studies were conducted of the effect of the wavelength of maximum absorbency (nm) distributed in the range between 400 and 700 nm and, the concentration of the dye (mg/L) between 62,5 and 250 mg/L. The selected interval of dye concentrations had no interference with the toxicity tests. To sum up, 13 experiments were carried out according to the experimental design based on **Table 1**.

Implications of the wavelength variable of maximum absorbency

The research of effects associated to the reduction of the quantity of light available for photosynthesis processes required a search for an objective variable of importance in the light transmission phenomenon. Likewise, it was essential that it not be subjective (depending on the observer) as is the national designation of color (red, blue, yellow, etc.). Consequently, there was a selection of maximum absorbency wavelength as an objective variable in the prediction model. It was easy to measure, as well as possesses an associated numerical value, which eases the use of the static model.

In addition to the above, the lethal concentration (LD50) of the Red 40 dye was determined using algae for the purpose of making qualitative comparisons with the *Daphnias* toxicity test.

4.1.1 Inhibition of algae growth associated to total solids

Design of experiments

Analog to adverse effects generated by dyes in aquatic media, suspended solids inhibit light transmission to greater depths with influences on photosynthesis processes. The experimental design used as a response variable, the reduction of the rate of cell duplication of the *Selenastrum Capricornutum* organism and as a main variable, the total concentration of solids expressed mg/L in the range of 30 to 100 mg/L. The total number of experiments conducted were 7, using the Central Compound design.

The tests were conducted with an inert solid (Silicon Oxide) to ensure they would not be molecular but rather because of light passage inhibition, which is the parameter that affects algae growth.

4.2 Evaluation of the toxicity associated to the main dyes and pigments used in the Aburrá Valley Metropolitan Area

For the purpose of evaluating ecotoxicity some of the main raw materials used in the industrial sectors that are presented below, as well as the efficiency of treatments systems used in them, the *Daphnias* immobilization test was implemented according to the ISO 6341 of 1982 Standard.

TABLE 1. CONCENTRATIONS AND MAXIMUM ABSORBENCY LENGTHS USED

Concentration (mg/L)	250	288,8	156,3	62,5	156,3	23,66	62,5	156,3	250
Wavelength (nm)	**454**	**517**	**427**	**580**	**517**	**517**	**454**	**606**	**580**
Color									

Industrial sectors evaluated: Textile, chemical, tannery, graphic arts, wastewater treatment, paper, ceramic, metal-mechanic and food and drink sectors.

Dyes and pigments evaluated: Crimson Indigo, Red 40, Yellow N° 5, Basic Chrome Sulphate, Black Iron Oxide, Titanium Dioxide, Zinc Oxide, Rhodamine β, Copper Oxide and Red Iron Oxide.

Of the aforementioned industrial sectors, four companies belonging to the textile, paper, chemical and sanitary (wastewater treatment) sectors were selected. A program was developed for these companies for the applied monitoring of wastewater treatment systems (entrances and exits). The sample toxicity was analyzed using the *Daphnias* toxicity test, as well as the algae test.

5. RESULTS AND DISCUSSION

Toxicity model associated to color

The correlation polynomial obtained from the design of experiments, toxicity over algae associated to the maximum absorbency wavelength (A) and the concentration of the dye (B) is described by **Equation 1**

$$\begin{aligned}\text{Ln (Toxicity)} = &-(57.24471 + 0.21066\ \mathbf{A}) + (0.027246\ \mathbf{B}) \\ &-(4.33653\text{E-}5\ \ \mathbf{A}\ \mathbf{B}) - (1.79870\text{E-}4\ \mathbf{A}^2) - (6.48280\text{E-}6\ \mathbf{B}^2)\end{aligned} \quad (1)$$

Table 2 shows the results of the variance analysis (ANOVA) for the design of the applied central compound

According to the analysis of results of the previous variance, the value of the Fischer (F) proof for the model (649,81) indicates it is significant. The values for "Probability > F" which are lesser than 0,0500 signal the significant terms of the model, for this case they are A, B, AB, A^2. Values above 0.1000 indicates the terms of the mode that are not significant. The deficiency of the adjustment for the value F (2,17) is not significant in relation to the pure error. There is a 23.47% probability that the lack of adjustment for value F will be large. This might occur because of the noise included for the value of each variable.

The R^2 predicted is in accordance with the adjusted R^2 of 0.9963. That is, the difference is less than 0,2.

The appropriate precision measures the relationship signal-noise. A proportion greater than 4 s desirable. The value of 74.495 indicates an adequate signal. This model may be used to predict the defined range for the main variables.

According to the information obtained by means of the ANOVA analysis of the results of the experiment design, the model of toxicity by color can predict 99% of the responses using as main variables the maximum absorbency wavelength (between 400 and 610 nm) and the concentration of the dye. The method cannot predict wavelength values greater than 610 nm due to the fact there are interferences on the algae growth reading for values close to 670 nm.

The lethal concentration, LD50 of Red 40 on the *Selenastrum Capricornutum* organism was 12,43 ppm.

Figure 1. Surface of response experiment designs: Toxicity by color

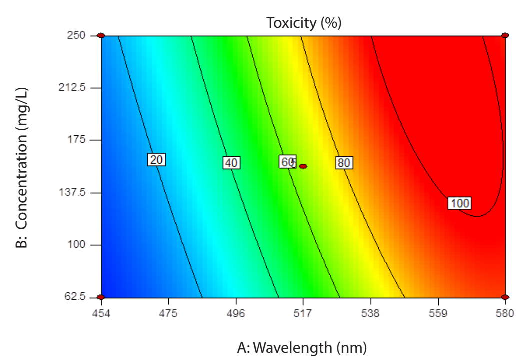

TABLE 2. ANOVA RESULTS – EXPERIMENT DESIGN OF TOXICITY BY COLOR

Source	Sum of Squares	Degrees of Freedom	Mean Squared	Value of F	Value p Prob > F
Model	14.54	5	2.91	649.81	< 0.0001
A: Wavelength	10.17	1	10.17	2272.99	< 0.0001
B: Concentration	0.55	1	0.55	123.20	< 0.0001
AB	0.26	1	0.26	58.63	0.0001
A^2	3.55	1	3.55	792.17	< 0.0001
B^2	0.023	1	0.023	5.05	0.0595
Residual	0.031	7	4.476E-3		
Adjustment error	0.019	3	6.464E-3	2.17	0.2347
Pure error	0.012	4	2.984E-3		
Corr. Total	14.57	12			

Standard deviation	0.067	**R^2**	0.9979
Average	3.71	**R^2 adjusted**	0.9963
C.V. %	1.80	**R^2 predicted**	0.9893
PRESS	0.16	**Adjusted precision**	74.495

TABLE 3. ANOVA RESULTS TOXICITY EXPERIMENT DESIGN FOR TOTAL SOLIDS

Source	Sum of Squares	Degrees of freedom	Mean Squared	Value of F	Value p Prob > F
Model	7.015E-4	1	7.015E-4	120.74	0.0001
A-Solid Totals	7.015E-4	1	7.015E-4	120.74	0.0001
Residual	2.905E-5	5	5.810E-6		
Adjustment error	2.905E-5	3	9.684E-6		
Pure error	0.000	2	0.000		
Corr. Total	7.306E-4	6			

Standard Deviation	0.9602	R^2	0.9602
Average	0.9523	R^2 adjusted	0.9523
C.V. %	0.9408	R^2 predicted	0.9408
PRESS	19.382	Adjusted precision	19.382

Toxicity model associated to total solids

Equation 2 presents the statistic model obtained after conducting a variance analysis of the results of experimental design of total solids. The model predicts the effects associated with total solids. It predicts the effects associated with the presence of total solids (A) in wastewater on the *Selenastrum Capricornutum* organism.

$$(\text{Toxicity})^{-1/2} = 0.17292 - 6.24297E\text{-}4\ \mathbf{A} \qquad (2)$$

Table 3, shows the development variance analysis for the experimental design applied.

The value F for the model was 120.74, which indicates that it is significant. The predicted R^2 (0.9408) reasonably agrees with the adjusted R^2 (0.9523). That is, the difference is less than 0,2. The appropriate precision measures the signal-noise. A proportion greater than 4 is desirable. The value of 19.382 indicates an adequate signal. This model can be used to predict the defined range for the main variables.

According to the information obtained by means of the ANOVA analysis, the toxicity model for total solids can predict 94% of the responses using, as a main variable, the concentration of total solids.

The algae growth inhibition trial was applied to determine the lethal dose (LD50) of solids in a water sample containing silicon oxide. The result obtained was that with a solid concentration of 48,683 ppm, half the algae growth is inhibited.

Ecotoxicity of some raw goods used by the industrial sectors of the Valle de Aburrá Metropolitan Area

Table 4 presents results of the application of the Daphnias toxicity test on wastewater containing some of the raw goods most used by the industrial sectors of the Valle de Aburrá Metropolitan Area.

TABLE 4. DAPHNIAS TOXICITY TEST RESULTS APPLIED ON MAIN RAW GOODS OF THE ABURRÁ VALLEY METROPOLITAN AREA

Dye	24h LD50 (ppm)	48h LD50 (ppm)
Crimson Indigo	---	970
Copper oxide	200	25
Yellow iron oxide	1550	1200
Zinc oxide	860	92
Black iron oxide	11000	5000
Red iron oxide	8000	7000
Green chrome oxide	6000	4700
Titanium dioxide	8700	8700
Red 40	10000	700
Yellow vegetable aniline	5800	5650
Rhodamine β	65	57

From the above results, it is possible to affirm, taking into account the regulatory framework that establishes the use of the toxicity test Daphnias (Resolution 062 of 2007), that effluents contaminated with Rhodamine β generate a greater toxicity on aquatic ecosystems. It is important to note that the Daphnias test is associated with the toxicity produced by the chemical nature of the molecule.

In addition, **Table 4** shows that the increase of exposure time reduces the LD50 value, as multiple authors express.

When comparing LD50 results obtained for Red 40, through the Daphnias toxicity test (700 ppm) and the algae test (12,43 ppm), the effect of the nature of both toxicities can be appreciated. That is, the toxicity of the constitutive molecule of the dye and those generated by the reduction in the quantity of available light for the development of photosynthesis processes.

Effluent toxicity of sectors: textile, chemical, paper and sanitary (wastewater treatment)

The toxicity of samples of wastewater (at entrance and exit points of the treatment systems) was analyzed from four of the most representative of the previously selected manufacturing sectors. **Table 5** depicts the characterization of the evaluated effluents.

TABLE 5. PROPERTIES OF SAMPLES FROM COMPANIES

Sector	pH	Redox (mV)	Conductivity (µS/cm)	Total Solids (ppm)
Chemical **	8,86	174,9	186,8	2695
Paper *	7,43	188,1	829	1989
Paper **	7,25	-31,6	909	1186
Wastewater treatment *	7,25	49,0	1239	1232
Wastewater treatment **	7,47	61,8	1150	713
Textiles *	12,40	32,5	5,72	3662
Textiles **	9,68	46,4	2,23	2162

*Entrance to treatment system ** Exit from treatment system

TABLE 6. RESULTS OF TOXICITY TESTS WITH ALGAE AND DAPHNIAS ON EFFLUENTS OF WATER TREATMENT SYSTEMS OF SELECTED COMPANIES

Sector	Daphnias 24h EC50	Daphnias 48h EC50	Algae 72h EC50
Chemical **	8%	6%	---
Paper *	20%	12,5%	---
Paper **	68%	50%	---
Wastewater treatment *	25%	17%	2,7%
Wastewater treatment **	73%	59%	6%
Textiles *	10%	5%	1,74%
Textiles **	38%	28%	3,5%

*Entrance to treatment system ** Exit from treatment system

The results of lethal doses at 24 hours for Daphnias indicate high levels of toxicity in the wastewater discharges in these sectors. The lesser the percentage of LD50, the greater the toxicity it represents. That is, with a small quantity of contaminator, a lethal effect is achieved for half the population. Under this criteria, we can state that the chemical and textile sectors present the most lethally toxic wastewater. In second place is the paper sector and lastly, the wastewater treatment sector.

The toxicity of the chemical sector was only determined for the exit of the treatment system. Because of the strong dark dye the sample contained, even with the respective dilutions, it caused interference with the toxicity test.

Like the chemical sector, the textile sector had very high toxicity values. Including at the exit of the treatment plant, toxicity remains high.

The paper and wastewater treatment sectors presented a lesser toxicity value at the exit of their respective treatment systems. However, their discharges remain toxic.

The result of lethal doses at 48 hours for the Daphnias show lethal dose values even lower. That is, after 48 hours of contact with the toxic compound, the mortality of half the population is obtained from low concentrations. This represents a toxicity grade when comparing this color with the one reported at 24 hours of the evaluation.

In the case of algae, it is even more critical. The results indicate algae is susceptible to this wastewater. All samples presented toxicity levels that were quite high. For example, in the case of the textile industry, the water before the treatment plant has a lethal dose of 1,74%. That is, if this volume of contaminated water were diluted in 1,74% of distilled water, it would cause an algae growth inhibition of 50% of the algae.

Exit samples of treatment systems of the textile and sanitary sectors showed significantly high toxicity values (3,5 y 6%).

The algae test for the chemical and paper sectors showed that effluents at the entrance and exit of treatment systems are highly toxic. The LD50 cannot be obtained because, for all dilutions evaluated, the result was a mortality of the entire organism population.

6. CONCLUSIONS

Prediction model of adverse effects generated by dyes and total solids on aquatic organisms

According to the results obtained, statistical models can be used for the prediction of effects associated with dyes and total solids in wastewater. However, their implementation is restricted to the wavelength of maximum absorbency (applied for values < 610 nm), as well as effects associated with molecular toxicity, which can be estimated from the *Daphnias* toxicity test.

Toxicity of raw goods used for leading industrial sectors of the Aburrá Valley Metropolitan Area

The raw goods with the greatest negative effects (molecular toxicity) on aquatic organisms, evidenced from the Daphnias test, were effluents containing Rhodamine β and, in lesser proportion, those who contained copper oxides. It was proven that lethal doses (LD50), gradually decrease with exposure time. Iron oxides generally presented greater LD50 values according to the Daphnias toxicity test.

Upon analyzing the behavior of soluble substances (dyes such as Red 40), it is found that the molecular toxicity values (LD50) were in the order of a magnitude 60 times greater to the toxicity value associated to the capacities these substances have to reduce the transmission of light. According to multiple authors, this is the result of sensitivity of the algae to the quantity of light available to their photosynthesis processes.

Toxicity evaluation of effluents of treatment systems of paper, chemical, sanitary and textile companies

Taking into account results obtained after applying the Daphnias and algae toxicity tests, we can observe a reduction in the toxicity grade of entrance effluents of treatment systems. However, dumping, according to the risk qualification protocol implementing contamination by toxicity index, ICOTOX, chemical and textile sector dumping is rated as toxic, while paper and sanitary sector dumping is rated as moderate. That is, that discharges belonging to these sectors, are currently potential generators of toxicity in the Aburrá – Medellín River, having a greater impact on photosynthesis organisms.

In general, it was possible to prove the effectiveness of the Daphnias and algae tests for determining molecular toxicity and associated to the color of the main raw goods and dumping from some industrial sectors of the Metropolitan Area of the Aburrá Valley.

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