Evaluation of the behavior of constitutive heterochromatin in chromosomes of Astyanax altiparanae (Pisces, Characidae) when submitted to the product of fungal remediation of textile dyes.

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Abstract

Specimens of Astyanax altiparanae were analyzed concerning the mutagenic potential of textile dyes Reactive Black 5 and Reactive Blue 19 and their products remediated by endophytic fungi, lineage Phlebias sp. through karyotype analysis in order to detect possible chromosomal alterations and variations on heterochromatin through band C. Twenty-eight copies of Astyanax altiparanae commercially obtained were submitted to different treatments being distributed 2 individuals each tank with 10L of water: tank 1 only with water (negative control); tank 2 with potato dextrose broth; tank 3 with dye Reactive Blue 19; tank 4 with dye Black 5; tank 5 with environment fermented by fungi and potato dextrose broth; tank 6 with environment fermented by fungi added with dye Reactive Blak 5. All solutions of treatment were made with concentration 0,01%/L and each treatment lasted for 24h. Metaphases of A. altiparanae revealed chromosomal breaks and also variations on distribution and quantity of heterochromatin among different types of treatment. The tested textile dyes seem to exert genotoxic effect due to Gaps and chromosomal breaks as well as epigenetic effect, due to alteration of chromatin compaction of analyzed specimens.

Introduction

The textile industries consume big volumes of water and chemical products on their processes of transformation. The liquid effluents of textile industries are typically colored due to extensive use of dyers in the processes of tinging and printing (Nigam et al., 2000). In liquid effluents treatment, dyes degradation is difficult given the complexity of molecular structure and its synthetic origin. More than 90% of 4,000 dyes tested by Ecological and Toxicological Association of the Dyes Tuffs Manufacturing Industry (ETAD) presented high values of toxicity. The highest rates were found among the azo basic and direct dyes (Robinson et al., 2001).

It is estimated that nearly 20% of world production of dyes are lost to environment during synthesis, processing or application that, if not properly treated, may reach reservoirs and water treatment stations besides ecological damages (Konstantinou e Albanis, 2004). The textile industries waste, constitutes by high concentrations of organic material, particulate material, pigments and dyes may provoke besides visual pollution, contaminations in water bodies, alterations in biological cycles affecting mainly the photosynthesis processes (Meriç et al., 2004). Stability of dyes to light depends not only on factors related to the material on which they are applied, but also to the incidence of radiation energy and to the facility with which the intermediaries are formed (Kuramoto, 1996). Moreover, studies have shown that azo dyes and their subproducts may be carcinogenic and/or mutagenic (Kunz et al. 2002). Such toxicity is related to liberation in the organism of toxic compounds coming from biological hydrolysis of azo bond (-N = N-). These free compounds, mainly aromatic amines, are suspect of carcinogenesis and mutagenesis (Umbuzeiro et al. 2005).

Azo-dyes are aromatic compounds, characterized by one or more grouping azo (-N = N-) (Konstantinou e Albanis, 2004; Mansour et al., 2007). These dyes are widely used on nylon and polyester tinging

(Guaratini e Zanoni, 2000). The great worry related to azo dyes is due to their polluting and toxic effects, and to big resistance to degradation (Zollinger, 1991; Arslan et al. 1999; Robinson et al., 2001). Many studies show that synthetic dyes are capable of causing damages to DNA (Ferraz et al., 2011; Umbuzeiro et. al, 2005; Chequer et al., 2009). Despite the great number of studies with these compounds, there are no enough data on literature to estimate the genotoxic risk of most synthetic dyes used nowadays.

This way, the releasing of azo dyes in the environment produces a potential risk of harm to health, once, when in water environment, the microorganisms may disintegrate it, releasing substances. Bearing in mind that there is more than 3,000 compounds of the azo dye types available for many manufacturing sectors, there is the need of performing the evaluation of toxicity of each dye individually, once each molecule can change its toxic proprieties (Umbuzeiro et al., 2005).

The search for sustainability is recurring not only for the textile industry, but also for other manufacturers of different areas. Reducing the consumption of hydric resources is one of the most explored issues, mainly in textile dyeing, where water consumption rate is very high.

Textile industry in particular presents an elevated demand of water in its processes, thus generating a big amount of residual waters, which, usually, have high amounts of dissolved salts, surfactants, suspended solids and organic matter, mainly the dyes in the form of complex molecules (Neamtu, 2002).

During last decades research has been made for new methods to make possible a new efficiency on the treatment of textile effluents in order to reduce their impact when they are discharged in water bodies and even the reutilization of the water contained in it. An alternative has been the use of biological degradation performed by microorganisms. Such processes are efficient in treating effluents, mainly by removing organic matter, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC). This degradation may be aerobic, where oxygen is the final acceptor of electrons, or anaerobic, where the final electron acceptor are inorganic ions (Sottoriva, 2006).

The bioremediation has proven to be an innovative technique in the process for degradation, elimination and inactivation of toxic waste. Microorganisms may be found in the very impacted environment, and they are, most of the times, the responsible of the contaminants´ disappearance (Oliveira e França 2005). The use of fungi for the degradation of organic or inorganic compounds has been occurring for some time in processes of biological treatment in water effluents and solid waste. The use of endophytic fungi is a bioremediation proposal for the toxic influence of textile dyes.

The present study performed an evaluation of the genotoxicity potential in the dyes Reactive Black 5 and Reactive Blue 19, as well as the products degraded by endophytic fungi, lineages of microorganisms, *Phlebia* sp. belonging to a collection of microorganisms from the Microbial Technology Lab of the State University of Maringá (Universidade Estadual de Maringá), in specimens of *Astyanax altiparanae.* This analysis involved tests of chromosome aberrations using the common technique for obtaining the metaphases aiming at detecting possible chromosomal mutations in the analyzed metaphases. Besides,

the analysis of the C-banding has as its aim to identify variations in the distribution trend and the amount of constitutive heterochromatin in specimens of *Astyanax altiparanae* receiving different treatments.

Materials And Methods

Animals

In this study, 28 individuals from the *Astyanax altiparanae* bought from the fishing shop Isca Viva in the city of Maringá, Paraná state were used.

Endophytic Lineage and Culture Conditions

The fungi lineage used in this study belongs to the specimen *Phlebia*s sp. from the microorganism collection of the Microbial Biotechnology Lab of the State University of Maringá (Universidade Estadual de Maringá).

The endophitic fungi was cultivated in dishes containing culture media Potato-Dextrose Agar (PDA) in pH 6,8. All essays were performed after 7 days of mycelial growth, a dish 6mm large was inoculated to the culture media of Potato-Dextrose (PDA) for the analyses.

For the samples containing dyes, 0,1 $g.L^{-1}$ from those were added in the culture media. The samples with Potato-Dextrose (PDA) in pH 6,8, were added the same concentration, 0,1 g/L in 10 L of water.

Features of the Dyes

Two kinds of dye were used in this study, one of them having the azo dye Reactive Black 5 (RB5), and the other one having an anthraquinone dye Reactive Blue 19 (RB19). The Reactive Blue 19 dye has the azo group as a chromophore, and it is characterized as an anionic dye (Image 1). The Reactive Black 5 dye belongs to the class of reactive dyes and has in its chemical structure two bindings from the azo type (Image 2).

Reactive Blue 19 (Sigma - Aldrich®)

Synonym: RemazolBrilliant Blue R

Molecular formula: $C_{22}H_{16}N_2Na_2O_{11}S_2$

Purity: ~ 50%

Molecular weight: 626.54 g/mol

CAS: 2580-78-1

Reactive Black 5 (Sigma - Aldrich®)

Synonym: Remazol Black B

Molecular formula: $C_{26}H_{21}N_5Na_4O_{19}S_6$

Purity: ~ 55%

Molecular weight: 991.82 g/mol

CAS: 17095-24-8

Animal treatments

The decontamination treatment was executed on 28 fish, of which 14 were used in the first test and one repetition with same concentration for the second test, distributed in a proportion of 2 fish per aquarium, during twenty four hours for each treatment as described below:

- Aquarium 1: 2 fish in an aquarium containing 10L of water (control individuals);
- Aquarium 2: 2 fish in an aquarium with Potato-Dextrose gruel in a concentration of 0,01%/L diluted in 10 liters of water.
- Aquarium 3: 2 fish in an aquarium containing Reactive Blue 19 dye (Sigma - Aldrich®) in a concentration of 0,01%/L diluted in 10 liters of water;
- Aquarium 4: 2 fish in an aquarium containing Reactive Black 5 dye (Sigma - Aldrich®) in a concentration of 0,01%/L diluted in 10 liters of water;
- Aquarium 5: 2 fish in an aquarium half fermented by *Phlebia*s sp. fungus and with Potato-Dextrose gruel in a concentration of 0,01%/L diluted in 10 liters of water;
- Aquarium 6: 2 fish in an aquarium containing Reactive Blue 19 dye (Sigma - Aldrich®) bio remediated by the *Phlebia*s sp fungus in a concentration of 0,01%/L diluted in 10 liters of water;
- Aquarium 7: 2 fish in an aquarium containing Reactive Black 5 dye (Sigma - Aldrich®) bio remediated by the *Phlebia*s sp. fungus in a concentration of 0,01%/L diluted in 10 liters of water;

Cytogenetic Analysis

Induction of the number of metaphases: a technique initially described by Cole and Leavens (1971) for amphibious and reptiles and adapted by Oliveira et al. (1988) for fish was used in the induction of the number of metaphases. The technique consists in previously injecting a suspension of biological ferment in the dorsum-lateral region of the fish in a proportion of 1ml to every 100g of weight of the animal. Leave the fish in a well aired aquarium for 24 hours.

Obtaining mitotic chromossomes

The preparations of mitotic chromosomes were made by using the cephalic portion of the kidneys, in accordance to the technique described by Bertollo et al. (1978). This technique consists, basically, in

treating the animal with colchicine (0,05%), sacrifice-it in an hour later and obtaining the cellular suspension of the cephalic kidney, hypotoned with KCL at 0,075 M and fixed in a solution of methanol and acetic acid (3:1). After the preparations, blades will be made by dropping 3 or 4 drops of the cellular suspension, which will be dyed with Giemsa (5%) for conventional analysis, or treated according to banding techniques.

Detection of constitutive heterochromatin (Band C)

The band C pattern was determined by applying Sumner's (1972) technique, in which the chromosomes should be treated with HCL 0,2N for 15 minutes, Barium (5%) for 1: 30 minutes and 2 x SSC for 60 minutes at a temperature of 25ºC, 40ºC e 60ºC, respectively. After the treatment, the blades were dyed with propidium iodide, according to Lui et al. (2009), the proportion of the mounting medium DABCO + 1µL of MgCl2 50mM + 1µL of propidium iodide solution, (50µg/mL); or 1 µL DAPI (2µg/mL) for each blade. Finally, an analysis is made of the blade using a fluorescence microscope. Both types of dyeing were used in this study.

Análise das lâminas

The Giemsa dyed blades were examined under an optical microscope of 400 or 1000 fold increase, and the total number of viable metaphases were evaluated for each treatment and control. Cell viability was performed using at least 80% of the total number of metaphases found. The blades dyed with propidium iodide were analyzed in fluorescence Zeiss Axioshosp light microscope with image capture. The captured images were processed with the help of Adobe Photoshop.

Results And Discussion

Reactive dyes are used on a large scale in the dyeing of cotton and viscose fibers, primarily for the clothing segment in medium and dark color intensity, due to its good levels of fastness to wet treatments. However, this type of dye reacts not only with the substrate, as well as water and thus ends up being a major constituent of dyers effluents.

Some studies such as Rivera et al. (2009) combined the conventional physical-chemical treatment with a technique called sono-electrochemical (the result of the combination of electrochemistry with ultrasound technology to improve the efficiency of treatment of textile effluents). The discoloration obtained after 1 hour of treatment was of 95%. One of the dyes used for the study was C.I. Reactive Black 5 (RB5) and the dye C.I. Reactive Blue 19 (RB19), dye also investigated in this work.

It is known that the photocatalytic ability of a semiconductor, under illumination, increased the degradation rate of an organic compound, which is attributed to the electronic structure, the pairs of produced electrons can recombine and dissipate energy as heat, or migrate to the semiconductor surface and participate in oxidation-reduction interfacial reactions through the formation of the hydroxyl root OH and superoxide anion root $O2^{-}$. This superoxide root anion in the presence of H2O is converted into

hydroxyl root which degrades the dye. Lucilha and Takashima (2009). Due to this fact the individuals (Astyanax altiparanae sp) studied were exposed in aquariums containing dyes for only 24 hours, because it is believed that after this time certain dyes would have been degraded. However when the fish was exposed for 48 hours it did not resist and died, a fact that may be an indication that dyes cause a real toxicity, affecting the fish lethally. The concentration of 0.01% of dye was chosen as this is an initial qualitative research.

The diploid number found for Astyanax altiparanae copies analyzed was 2n = 50 being consistent with this species and the karyotype formula was 8m + 20sm + 14st + 8a (Image 3). Although the diploid number of 50 chromosomes is maintained in all populations of A. altiparanae analyzed to date, different karyotypes formulas have been described (Fernandes and Martins-Santos, 2004; Pacheco et al., 2011). As this species NF varies between 76 and 100 (Fernandes and Martins-Santos, 2004), is possible to observe very divergent and other relatively close formulas, suggesting that small differences are related to the criteria adopted in the classification of types of chromosomes. As an example, the structure presented by *A. altiparanae* specimens (8m + 20sm + 14st + 8a) of the present study approaches the formula described for the Parana River population analyzed by Fernandes and Martins-Santos (2004) which is composed of 6m + 26sm + 6st + 8a. Perhaps if we conduct a karyotype reorganization it might suggest the same constitution for all individuals, whereas the copies of this study were obtained commercially and can be obtained from the Paraná River.

Image 3. Karyotype Giemsa of *Astyanax altiparanae.* 2n = 50;

The pattern of constitutive heterochromatin observed in this study (Image 4) for individuals untreated (negative control) showed few heterochromatic blocks in the species, highlighting some pericentomeric, telomeric and interstitial markings. Heterochromatic markings were common in pairs of chromosomes 3, 4 (metacentric), 7, 8 (submetacentrics), 15, 16 and 19 (subtelocentric).

In individuals undergoing solution containing BDA broth (Potato Dextrose, aquarium 2) the standard C band was equal to that of control subjects (1 aquarium). On the other hand in individuals treated with Reactive Blue 19 dye (blue dye, aquarium 3) additional heterochromatic marks were observed in pairs 6, 13, 14 (metacentric) and 22 (acrocentrics). In specimens treated with Reactive Black 5 dye (black dye, aquarium 4) there was a decrease in the distribution of heterochromatin on the chromosomes, not being observed bands on the pair 8. In the treatment of fish with BD broth and fermented medium by the fungus Phlebias sp. (Aquarium 5) pairs marked by C band were similar to fish pairs treated with Reactive Blue 19 dye, except for pairs 16:18 (sm) and acrocentric 22:25 (Image 4).

The subjects of the aquarium (6 and 7) containing the product solution remediated by fungus have also shown a heterochromatic pattern with slightly differences concerning the control: aquarium 6 (reactive Black and medium fermented by the fungus) differed from the control in the pairs 18 and 25, while aquarium 7 (reactive Blue and medium fermented by the fungus) differed from the control in the pairs 18 and 25 3 (See Image 4). When comparing the treatment results of the aquariums 3 and 6, it is possible to observe an increase in the quantity of pairs marked by the heterochromatin in fishes treated with the

pigment reactive Blue in relation to the submetacentric pairs; whereas in aquarium 6 there was a decrease in the number of chromosome pairs with heterochromatin.

At first, these results suggest a bioremediation effect by the Phlebias sp. fungus in aquarium 6. However, through the same comparison, the aquariums 4 and 7 showed two additional pairs with heterochromatin in the aquarium containing the pigment reactive Black 5, and the product bioremediated by the fungus is not the same ones observed in aquarium 4, which contained only this pigment. The pairs 18 and 25 were observed to have heterochromatin only in fishes treated through the medium fermented by the fungus the Phlebias sp, being considered chromosomal markers in the presence of the fermentation. Likewise, the pairs 6, 13, 14 and 22 can be considered as biomarkers concerning the presence of the pigment Reactive Blue 19.

Recent studies have shown that heterochromatin variations regulate the gene expression epigenetically through the reversible transformation between the heterochromatin (noncoding sequences) and the euchromatin (coding sequences) (Hong et al., 2011). In frogs of the gender Litoria, a large variation in the C bands' distribution pattern in secondary constriction sites seems to be related to the transformation of euchromatin into heterochromatin (King, 1980). In fishes of the gender Astyanax, the heterochromatinization hypothesis of euchromatic regions to explain the variation of the C bands distribution pattern was also considered by Mantovani et al. (2000) and by Hashimoto and Porto-Foresti (2010).

In some species of Astyanax, variations in the quantity and distribution of heterochromatin have been confirmed, as registered in Astyanax scabripinnis by Mantovani et al. (2000) and in A. bockamanni by Hashimoto and Porto-Foresti (2010). Several hypotheses were considered for explaining the polymorphism in the heterochromatin distribution, such as: heterochromatin transference between sites equidistant from the centromere of non-homologous chromosomes during anaphase (Souza et al., 1996); heterochromatinization of euchromatic regions (King, 1980); unequal crossing-over (Smith, 1976); amplification, accumulation and elimination (John, 1988) of heterochromatic segments and presence of transposable elements associated to heterochromatin (Slotkin Martienssen, 2007).

The different toxic substances present in the rivers' waters are capable of generating epigenetic effects, besides provoking damages in the DNA level. Several epigenetic mechanisms, including DNA's methylation, modification of histones and micro RNA expressions, can be altered under the influence of exogenous agents such as environmental pollutants (Baccarelli; Bollati, 2009). These alterations in the epigenetic marks result in conformational changes of the chromatin. In Triatoma infestans, for example, the action of drugs like trichostatin A (TSA) and sodium butyrate (NaBt) promoted the heterochromatic decompression in these insects (Alvarenga, 2011).

In addition to the alterations in the heterochromatin distribution patterns observed in this study, chromosomal gaps and breaks were seen in some metaphases of the animals treated with the pigment Reactive Black 5 (See image 6). Several pollutants can be associated with the induction of chromosomal aberration of various fish species, both in natural and experimental conditions (Al-Sabti, 1991). The

aquatic environment is the last receptor of the pollutants produced by natural and anthropic sources, and their bioaccumulation and persistency constitute a threat to the biological life. Many pigments, although submitted to a toxicological evaluation previously to their use, have shown to be potentially ecotoxic, genotoxic and/or mutagenic in innumerous works of literature (Ferraz et al., 2011; Oliveira et al., 2010; Vacchi et al., 2013).

In the present study, the textile pigments tested also seem to cause such genotoxic effect due to chromosomal gaps and breaks found (See image 5 and 6) as epigenetic effect, in accordance with the alteration in the chromatin compression (See image 4). The data obtained for the heterochromatin's pattern variation in Astyanax paranae specimens constitute an important reference to the genotoxic potential of the pollutants in specific regions of the chromosomes. This can be used in other fish species as a model, which may be interpreted as a bioindicator species of polluted environments. Although the C band technique is a common methodology in cytogenetics, the information obtained from it represents an initial data of the alterations that may be complemented with other methodologies.

Image 4: Heterochromatin pattern of Astyanax paranae subjects submitted to different treatments: Control (aquarium 1); BD = Potato Dextrose (aquarium 2); Reactive Blue Pigment (aquarium 3); Reactive Black 5 Pigment (aquarium 4); BD and fungous medium (aquarium 5); product solution remediated by the fungous plus the Reactive Blue 19 pigment (aquarium 6) and product solution remediated by the fungous plus the Reactive Black 5 Pigment (aquarium 7).

Statements & Declarations

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References

1. Alvarenga EM (2011) Territórios heterocromáticos em *Triatoma infestans* Klug e. *Panstrongylus megistus* (Burmeister): composição, identificação de marcadores epigenéticos e resposta a inibidores de deacetilases de histonas. Dissertação, Universidade Estdual de Campinas,SP

2. Al-Sabti K (1991) Handbook of genotoxic effects and fish chromosomes. ISBN 8680023-17-5, pp. 230, *Josef Stefan Institute, Jamova* 39, 61111 Ljubljana, Slovenia
3. Arslan I, Balcioglu IA, Tuhkanem T (1999) Oxidative treatment of simulated dyehouse effluent by UV and near-UV light assisted Fenton´s reagent. Chemosphere 39:2767–2783
4. Baccarelli A, Bollati V (2009) *Epigenetics and environmental chemicals. Current Opinion in Pediatrics, 21(2):243 – 51.*
5. Bertollo LAC, Takahashi CS, Moreira-filho O (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Braz J Genet 1:103–120
6. Chequer FMD, Angeli JPF, Ferraz ERA, Tsuboy MS, Marcarini JC, Mantovani MS, Oliveira DP (2009) The azo dyes Disperse Red 1 and Disperse Orange 1 increase the micronuclei frequencies in human lymphocytesss and in Hep G2 cells. Mutat Res 676:83–86)
7. Cole CJ, Leavens CR (1971) Chromosome preparations of amphibians and reptiles: improved technique. Herpetological Rev 3:102
8. Fernandes CA, Martins-Santos IC (2004) Cytogenetic studies in two populations of Astyanax altiparanae (Pisces, Characiformes). *Herditas* 141.,328–332
9. Ferraz ERA, Umbuzeiro GA, De-Almeida G, Caloto-Oliveira A, Chequer FMD, Zanoni MVB, Dorta DJ, Oliveira DP (2011) Differential toxicity of Disperse Red 1 and Disperse Red 13 in the Ames test, HepG2 cytotoxicity assay, and Daphnia acute toxicity test. Environ Toxicol 26:489–497
10. Guaratini CCl, Zanoni MVB (2000) Corantes têxteis. Quim Nova 23(1):71–78)
11. Hashimoto DT, Porto-Foresti F (2010) Chromosome polymorphism of heterochromatin and nucleolar regions in two populations of the fish *Astyanax bockmanni* (Teleostei: Characiformes). Neotropical Ichthyol 8(4):861–866
12. Hong Y, Zhou Y-W, Tao J, Wang S-X, Zhao X-M (2011) **Do polymorphic variants of chromosomes affect the outcome of in vitro fertilization and embryo transfer treatment?** *Human reproduction*. England) 26(4):933–940)Oxford
13. John B (1988) The biology of heterochromatin. In: Verma, R.S. (Ed.). Heterochromatin: Molecular and Structural Aspects. *Cambridge*, *Cambridge University Press*
14. King M (1980) C-banding studies on Australian hylid frogs: secondary constriction structure and the concept of euchromatin transformation. Chromosoma 80:191–217
15. Konstantinou IK, Albanis TA (2004) TiO2-assisted photocatalytic degradation of azo dyes in aqueous solution: kinetic and mechanistic investigations. A review Applied Catalysis B: Environmental. 49:1–14)
16. Kunz A, Peralta-Zamora P, Moraes SG, Durán N (2002) Novas tendências no tratamento de efluentes têxteis. Química Nova 25(1):78–82
17. Kuramoto N (1996) The photodegradation of synthetic colorants. In: PETERS AT, FREEMAN HS (eds) Physicochemical Principles of Color Chemistry. Blackie Academicand Professional Publishers, London, pp 196–253

18. Lucilha AC, Takashima K (2009) Efeitos de agentes oxidantes e oxigênio dissolvido na descoloração do azo corante acid Orange 7 por fotólise e fotocatálise. Química Nova 32(6):1399–1404
19. Lui RL, Blanco DR, Margarido VP, Moreira-Filho O (2009) First description of B chromosomes in the Family Auchenipteridae, Parauchenipterus (Siluriformes) of the São Francisco basin (MG, Brasil. Micron 40:552–559
20. Mansour HB, Corroler D, Barillier D, Ghedira K, Chekir L, Mosrati R (2007) Evaluation of genotoxixity and pro-oxidant effect of the azo dyes: Acids yellow 17, violet 7 and orange 52, and of their degradation products by Pseudomonas putida mt-2. Food and Chemical Toxixology 45:1670–1677
21. Mantovani M, Abel LDS, Mestriner e Moreira-Filho CA, O (2000) Accentuated polymorphism of heterochromatin and nucleolar organizer regions in *Astyanax scabripinnis* (Pisces, Characidae): tools for understanding karyotypic evolution. Genetica 109:161–168
22. Meriç S, Kaptan D, Olmez T (2004) Color and COD removal from wastewater containing Reactive Black 5 using Fenton´s oxidation process. Chemosphere 54(3):435–441
23. Neamtu M (2002) Kinetics of decolorization and mineralization of reactive azo dyes in aqueous solution by the UV/H2O2 oxidation. Dyes Pigm 53:93–99
24. Nigam P, Armour G, Banat IM, Singh D, Marchant R (2000) Physicalremoval of textile dyes from effuents and solid state fermentation by dye-adsorbed agricultural residues. Biores Technol 72:219–226
25. Oliveira C, Toledo LFA, Foresti F, Britski HA, Filho T, S. A (1988) Choromosome formulae of Neotropical fresh water fishes. Revista Brasileira de Genética 11:577–624
26. Oliveira FJ, de França FP (2005) Increase in removal of polycyclic aromatic hydrocarbons during bioremediation of crude oil-contaminated sandy soil. Appl Biochem Biotechnol 121(124):593–603
27. Oliveira GAR, Ferraz ERA, Chequer FMD, Grando MD, Angeli JPF, Tsuboy MS, Marcarini JC, Mantovani MS, Osugi ME, Lizier TM, Zanoni MVB, Oliveira DP (2010) Chlorination treatment of aqueous samples reduces, but does not eliminate, the mutagenic effect of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. Mutat Res 703:200–208
28. Pacheco RB, Rosa R, Giuliano-Caetano L, Júlio HF Jr, e Dias AL (2011) Cytogenetic comparison between two allopatric populations of *Astyanax altiparanae* Garutti et Britski, 2000 (Teleostei, Characidae), with emphasis on the localization of 18S and 5S rDNA. Comp Cytogenet 5(3):237–246
29. Peralta-Zamora PG, Kunz A, Duran N, Moraes SG (2002) Novas tendências no tratamento de efluentes têxteis. Química Nova 25(1):78–82
30. Rank J (2003) The method of anaphase-telophase chromosome aberration assay. Ekologija 1:38–42
31. Rivera M, Pazos M, Sanroman (2009) Improvement of dye electrochemical treatment by combination with ultrasound technique. J Chem Technol Biotechnol 84:1118–1124
32. Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: acritical review on current treatment technologies with a proposed alternative. Bioresour Technol 7:247–255

33. Zhang R, Adams PD (2007) Heterochromatin and its Relationship to Cell Senescence and Cancer Therapy. 6:784–789. 10.4161/cc.6.7.4079. 7
34. Slotkin RK, Martienssen R (2007) Transposable elements and the epigenetic regulation of the genome. Nat Rev 8:272–285
35. Smith GP (1976) Evolution of repeated DNA sequences by unequal crossover. Science 191:528–535
36. Sottoriva PRS (2006) Remedição de efluentes têxteis por processos oxidativos avançados integrados a lodos ativados. Lorena, SP. Tese de Doutorado. USP, p 192
37. Souza IL, Moreira-filho O, Galetti JR, P.M (1996) Heterochromatin differentiation in the characid fish Astyanax scabripinnis. Revista Brasileira de Genética 19(3):405–410
38. Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304–306
39. Umbuzeiro GA, Freeman H, Warren SH, Oliveira DP, Terao Y, Watanabe T, Claxton LD (2005) The contribution of azo dyes to the mutagenic activity of the Cristaisriver. Chemosphere Oxf 60:55–64
40. Vacchi FI, Albuquerque AF, Vendemiatti JA, Morales DA, Ormond AB, Freeman HS, Zocolo GJ, Zanoni MV, Umbuzeiro G (2013) Chlorine disinfection of dye wastewater: Implications for a commercial azo dye mixture. Sci ofhe Total Environ t 442:302–309
41. Yu an, Jiang L, Cao J, Geng C, Zhong L (2007) Sudan I induces genotoxic effects and oxidative DNA damage in Hep G2 cells. Mutat Res 627:164–170
42. Zhang Y, Yu an, Jiang L, Geng C, Cao J, Jiang L, Zhong L (2009) The Role of Oxidative Stress in Sudan IV-Induced DNA Damage in Human Liver- Derived Hep G2 Cell. *Environmental Toxicology*, DOI 10.1002/ tox
43. Zollinger H (1991) Syntheses, properties and applications of organic dyes and pigments. Color Chemistry,New York: *V.C.H. Publishers*, 496

Figures

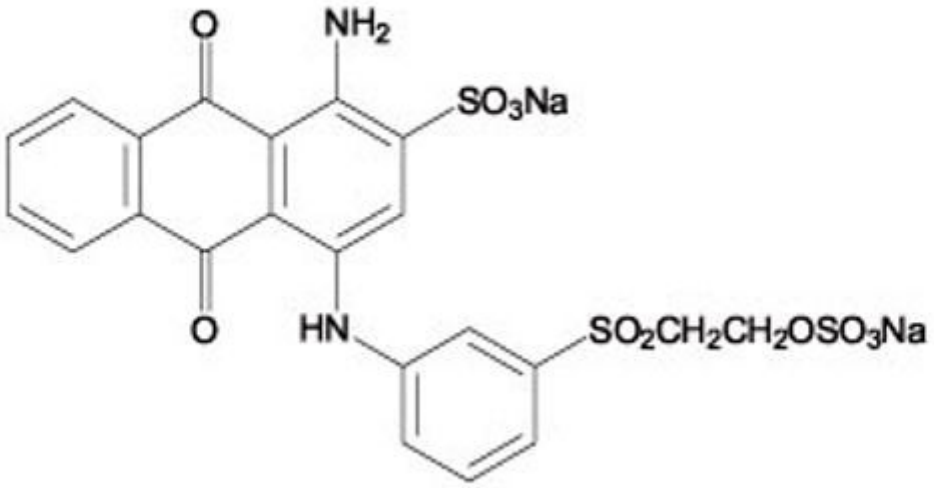

Figure 1

Reactive Blue 19 dye's structural formula

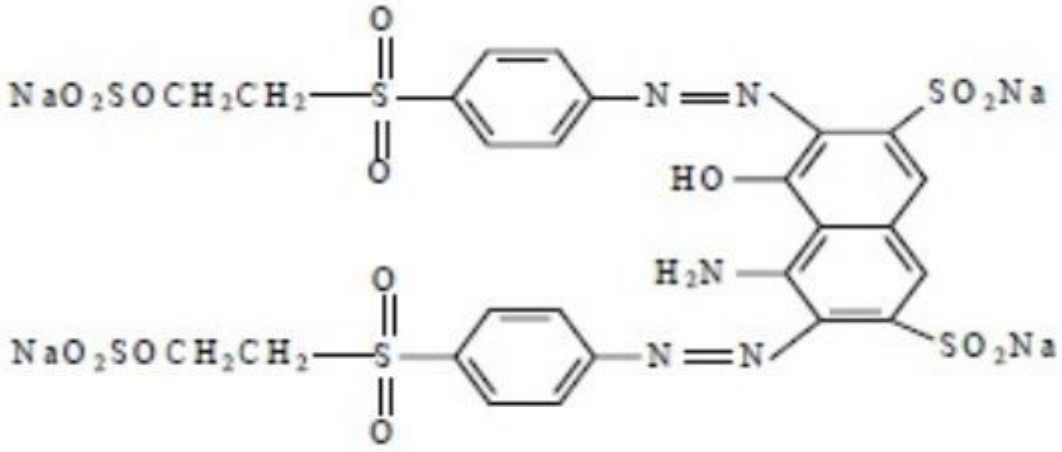

Figure 2

Reactive Black 5 dye's structural formula

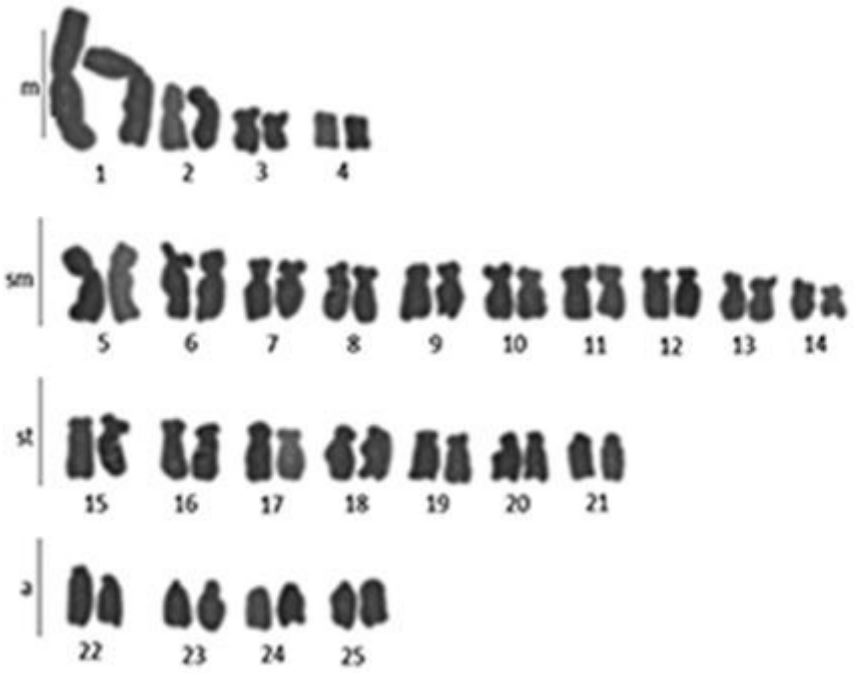

Figure 3

Karyotype Giemsa of *Astyanax altiparanae.* 2n=50;

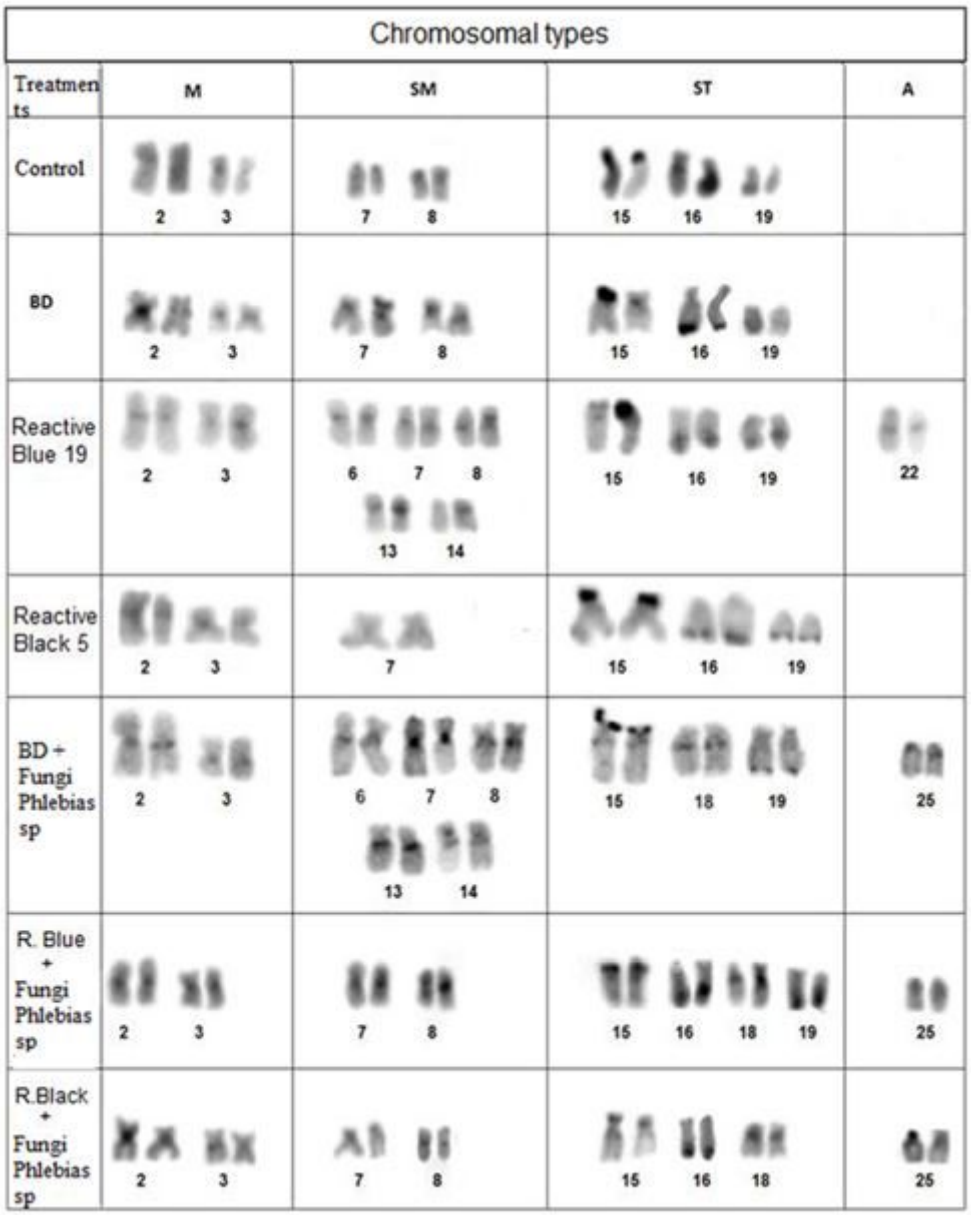

Figure 4

Heterochromatin pattern of Astyanax paranae subjects submitted to different treatments: Control (aquarium 1); BD = Potato Dextrose (aquarium 2); Reactive Blue Pigment (aquarium 3); Reactive Black 5 Pigment (aquarium 4); BD and fungous medium (aquarium 5); product solution remediated by the fungous plus the Reactive Blue 19 pigment (aquarium 6) and product solution remediated by the fungous plus the Reactive Black 5 Pigment (aquarium 7).