TRANSVERSAL TEACHING PROCESSES WHEN EVALUATING THE CYTOGENETIC EFFECT OF PARANITROPHENOL AND MALATHION IN THE SPECIES *Oreochromis sp* (RED TILAPIA): THE CASES OF NATURAL SCIENCES AND MATHEMATICS

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ABSTRACT

This dissertation is part of an academic research that is framed within the requirements of a master's thesis, as well as an undergraduate monograph, carried out by a group of students and professors linked to the Popular University of Cesar, specifically in the Faculty of Education. The study in question, of greater magnitude, had the fundamental purpose of evaluating the genotoxic effect of paranitrophenol and malathion on the blood cells of the species Oreochromis sp (Red Tilapia), through the implementation of the micronucleus test. Subsequently, this approach was taken up by teachers and students of the Bachelor's Degree in Mathematics, with the aim of developing transversal teaching processes, based on the case analyzed in the science classes. All of the above was carried out under an experimental design methodology, using the participatory action research model. The main findings of this study revealed, on the one hand, that when evaluating the genotoxic effect, it was determined that paranitrophenol, a highly relevant metabolite of methyl parathion, exhibits a significant genotoxic effect at concentrations of 10, 20 and 40 ng/mL, after a 48-hour exposure period in the species Oreochromis sp. On the other hand, it was evidenced that contextualized teaching, based on fish farming, generates high expectations in students, thus promoting significant learning that transcends the conventional academic environment.

1. INTRODUCTION.

The teaching of science has an important impact on the integral formation of the individual, as it leads the student to acquire new knowledge that complements their personal, cognitive and disciplinary development, as well as the development of creativity in the application of the concepts acquired, training individuals capable of solving problematic situations that their reality poses to them. The development of experimentation, for didactic purposes in secondary school students, implies the application of useful methods and techniques in the design of experiments and in the study of concrete phenomena or situations that students face on a daily basis. These techniques allow us to understand more clearly and confidently the usual procedures in the study of singular facts with small differences, living individuals and those obtained in very different conditions. The basis for the application of experimental research to the evaluation of the different disciplines of science is the fact that knowledge of universal methodological principles is dispensable for the deepening, understanding and logical analysis of biological phenomena. In addition, these same circumstances are applicable between the methodical links shared by the disciplines of the secondary school curriculum. In particular, the logical and methodological analysis of life processes makes it possible to illustrate the methods used in the constructions of atomic sciences and to draw parallels between their phases.

To carry out this research, the I.A.P (Action Participation Research) research method was necessary, which seeks to obtain reliable and useful results, basing the research on the active and formal participation of the researcher, where the intervention of both the researcher and the actors was encouraged in the achievement of the objectives of the project.

Anthropogenic activities have generated a wide variety of pollutants, and their effects depend on the concentration of the substances, their persistence and bioavailability, causing from non-lethal effects to the death of entire populations (Ramírez and Mendoza, 2008).

Rodríguez (2000) states "The department of Cesar is one of the departments of Colombia with the highest amounts of pesticides considered persistent environmental pollutants in the environment"

Among these compounds found in this department are malathion, methyl parathion and toxaphene. Paranitrophenol (PNP) is a metabolite of the pesticide methyl parathion, which is considered a mutacarcinogen.

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Exposure to pesticides can occur both directly and indirectly, through the digestive, dermal or respiratory tract, which puts at risk not only the people who apply the chemicals directly (Calva & Torres, 1998), but also those who consume products derived from agricultural activity (Muñoz-Quezada, Lucero, Iglesias & Muñoz, 2014), such is the case of infants who ingest it through breast milk. Early children represent one of the populations most susceptible to the effects of pesticides, associated with the fact that their biological capacities are developing and are required to metabolize the toxic substances that enter their system through different pathways (Zayas & Cabrera, 2007).

The effects of pesticides on health can be witnessed immediately after direct contact, as in acute pesticide poisoning, characterized by numbness, muscle pain, blurred vision, headache, disorientation, nausea and vomiting, and seizures and loss of consciousness may occur; When exposure to pesticides occurs chronically, even in low doses, the effects caused are cumulative, since diseases can occur that manifest themselves in the medium and long term, for example, the development of disorders of the liver, lungs, central nervous system, immune system as well as endocrine destabilization and cancer (Arrollo & Fernández, 2013; Benítez-Leite, et al., 2012; Conant & Fadem, 2011; Fernícola, 1985).

The micronucleus test is an easy technique to perform, and very useful to evaluate the impact of adverse factors on the genetic material of exposed organisms.

Micronuclei are cytoplasmic bodies of a nuclear nature, they originate from chromosomal breaks, errors during DNA replication and subsequent division and/or exposure to genotoxic agents. There are factors that can influence or modify the number of micronuclei present in a cell, such as: age, gender, medical treatments, daily exposure to an agent, etc. The genetic material that makes up micronuclei is mainly acentric chromosomal fragments, but it can also be derived from whole chromosomes. Zalacain, M; Sierrasesúmaga, L; Patiño, A (2005).

2. CONTEXT AND JUSTIFICATION OF THE RESEARCH

One of the great pending subjects in the development of competencies is in the areas of natural sciences and mathematics, which have historically resulted in extremely watertight disciplines. The associated disciplinary work logics prevent us from visualizing the relevance of the contents proposed by the neighboring area, diluting the possibility of generating differentiated and relevant learning. This problem arises from a reductionist vision of education; This position conceives the process as a product of simple aggregates of supposedly secure disciplines free of any axiological assumption. As such, the product is limited to the capture of partial impressions, thus enriching a supposed quality of cognitive conflict. Likewise, any answer that is not given in terms of observed evidence is undesirable, so everything is solved based on the problem of how both processes, one of learning and the other of teaching, are related.

Under this scenario, education faces the challenge of carrying out an open and critical synthesis between complex disciplines, because while it is true that, in fact, knowledge continues to fragment indefinitely, it is also true that knowledge coming from pure insignificance, fragmented and without links to the totality of human existence, is useless. Therefore, in diversifying knowledge, education must seek to unify the particular knowledge that would increasingly correspond to the constitution of scientific methods, in a global and harmonious knowledge that overcomes the dilemmas of everyday life, instructing for the whole man or woman and not for each of its aspects. An expression of global knowledge, scientific thought goes beyond its field of traditional hegemony, extending its postulates beyond the content of the systematic teaching of scientific disciplines.

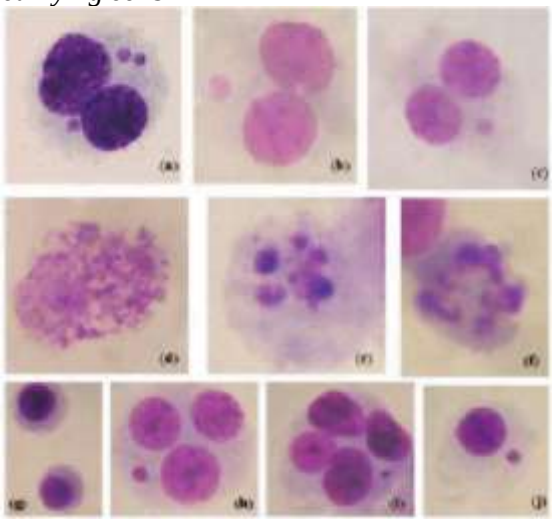
3. PROCESSES OF EVALUATION OF THE CYTOGENETIC EFFECT OF PARANITROPHENOL AND MALATHION IN THE SPECIES *Oreochromis sp* (RED TILAPIA)

The year 1999 was crucial for the MN assay, as the technique was validated worldwide and considered an effective biomarker of DNA damage. For the validation, an international human micronucleus program (HUMN: *HUman MicroNucleus Project*) was created, designed by Michael Fenech and Stefano Bonassi in order to collect the basal frequencies of NM obtained in different laboratories and populations around the world. The main objective was to identify the sources and levels of variability capable of influencing the basal frequency of micronuclei in human lymphocytes, to compare the different techniques used to define a standard protocol and thus carry out a prospective

study by all the laboratories involved and even to try to establish an association between the frequency of NM and diseases such as cancer.

Figure 1.

Binucleated micronucleus-carrying cells



Note. *(a) (b) and (c) binucleated cells carrying micronuclei; (d)(e) and (f) different stages of cells in the process of apoptosis; (g) (h) and (i) cells with different rates of nuclear division; (g) mononucleated cells; (h) trinucleated cell carrying micronuclei; (i)tetranucleated cell carrying micronuclei; (j)mononucleated cell with micronuclei.*

The use of the MN counting technique as a measure of chromosomal damage to human lymphocyte cultures was first proposed by Countryman and Heddle in 1976, whose only requirement was the choice of cell types with high mitotic activity. Later, in 1985, the genotoxicity assay was improved by Fenech and Morley, managing to slow down the process of cell division when the cell had only undergone mitotic division, for this they developed the *cytokinesis-block micronucleus* (CBMN) technique, which is based on the use of a chemical agent called cytochalazine-B whose function is to prevent cell cytokinesis.

Hooftman & de Raat (1982) adapted the MN technique to fish to assess the effects of exposure to carcinogenic and/or mutagenic substances (Zhu *et al*., 2004, Porto *et al*., 2005). Fish are considered to be ideal bioindicator organisms because they cover many links in the food chain, are capable of accumulating toxic substances, and react easily to low concentrations of mutagenic agents (Minissi *et al*., 1996; Gustavino *et al*., 2001). In addition, freshwater fish have more blood than saltwater fish, making them very useful in toxicology experiments when working with blood cells for monitoring purposes (Al-Sabti & Metcalfe, 1995).

3.1 Risk assessment

Human health risk assessment for chemicals is generally a study to estimate the likelihood of adverse health effects occurring in an individual, subpopulation, or population due to exposure to some chemical (heavy metals, for example). Risk assessment consists of four main steps: 1) hazard identification; (2) hazard characterization, including dose-response assessment; 3) exposure assessment and finally 4) risk characterization (UNEP, 2008).

3.2 Regulatory framework

Within the research context on principles of environmental law; Prevention, precaution and correction, Colombia is subject to some international agreements and treaties on environmental and health matters, such as:

Stockholm Declaration in 1972: "Man has the fundamental right to freedom, equality and the enjoyment of adequate living conditions in an environment of such quality as will enable him to lead a life of dignity and to enjoy well-being, and has a solemn obligation to protect and improve the environment for present and future generations" (WHO, 1972).

The Ottawa Charter: Which consists of a call to promote to the people the necessary means to improve

their health and exercise greater control over it. To achieve adequate physical, mental and social well-being, since health can be interpreted as a source of wealth for daily life, therefore the concept of health coincides as a transcendent well-being (WHO, 1986).

Brundtland Report in 1987: This report published by the United Nations seeks and contrasts the position of current economic development in the face of environmental sustainability, its influence on the well-being and health of populations (WHO, 1987).

The types of pesticides in use vary between countries and over time. In some developing countries, the trend is similar to that of industrialized countries, where a higher proportion of herbicides and fungicides are consumed. In contrast, in less developed countries, highly toxic insecticides continue to be the main agrochemicals in use (Wesseling et al., 1997). In three African countries (Tanzania, Kenya and Uganda) organochlorine compounds such as DDT, Dieldrin, Aldrin, Lindane and Campechlor continue to be used in food and feed crops, which are banned in most countries (Mbakaya, 1994). Of the 46 pesticide applications used in 1990 for cotton cultivation in Nicaragua, 26 of them were of methyl parathion, classified by the WHO as extremely hazardous (category Ia) (Murray, 1994). In these countries, the registration system as a strategy to manage risk has failed, so that their populations continue to be exposed to large quantities of highly toxic pesticides, even banned or severely restricted in developed countries (Duchastel and Walter, 1979).

The term pesticides encompasses insecticides, nematicides, herbicides, fungicides, rodenticides, growth regulators and fruit thinning agents (WHO/UNEP, 1990). This definition is equivalent to what some authors and environmental non-governmental organizations call agrotoxics. Even broader is the agrochemical concept, which in addition to pesticides or agrotoxins includes chemical fertilizers.

In the early 1960s, Carlson (1962) pointed out the wide environmental distribution of pesticides (in soil, air, water, biota, etc.), as well as their effects on human health associated with their indiscriminate handling and use. During the following decades, the chemical industry has continued to produce and launch on the market a large quantity and diversity of new active ingredients and formulations, which agricultural producers use intensively, without controlling the effects on the environment and human health.

3.3 Transversal Teaching in the Educational Context

- Transversal teaching processes allow analysis, inquiry, formulation of theory and verification of it in situations of reality. "The student has to inquire, reason and apply what they have learned instead of repeating concepts disconnected from reality. This method effectively transmits the value of scientific theories, because in a practical activity, when diagnosing a problem, theoretical solutions are offered, but guided by the need to change a specific aspect of reality." "Therefore, the student learns a scientific construction with an applicative sense, thus incorporating internal experiences, but always in correspondence with external practices that help him to design interiors of a more general nature. In this way, the application of the contributions of science to man should help him in the furrow of theoretical systematization." Similarly, it is ensured that transversal teaching can provide curricular coherence between the different areas of the school curriculum. In the same way, it can promote awareness among students that concepts and problems are interdisciplinary, since the knowledge of scientific facts is based on a multiplicity of logical relationships that can only be considered internally through simultaneous reference to various disciplines that make up the knowledge system. - Another way of defining transversal teaching in the educational context is the way in which various sciences pass their concepts to other subjects in order to provide broader information about them, so that students are awakened with an interest independent of the subject. Examples that we are taught today are the effect of pea rain or the biological origin of cellulose, hand in hand with the subjects of Knowledge of the Environment that include the basic content related to these issues.

4. METHODOLOGY

The experimental design that was implemented for this study was developed and applied taking into account the recommendations of the International Programme on Chemical Safety – IPC, and Palacio J. (2007) for in vivo genotoxicity studies in fish. The protocol to be followed was the one described by (Henao) 2008, with some modifications.

4.1 Participatory action research.

This type of research was framed in the environmental mutagenesis research line of the BIOTECGEN research group; the participatory action research model has been displaced from the positivist universe, such as that of values for the development, self-realization and emancipation of people, in contrast to the interests of subordination and domestication inherent in positivist scientific research. This derives from the ontological presuppositions embraced by this paradigm, which attest to the existence of a reality, independent of our consciousness, an objective dualistic position, as conceived by the positivist and post-positivist paradigms (Morse, 2000).

4.2 Technique to be used

4.2.1 Data collection strategy.

The strategies or methods used during the research were quantifiable data on which representative samples were taken as validation criteria. Data collections varied in this case according to the concentrations of the compound PNP and malathion that were evaluated in the groups of five fish/tank**.**

4.2.2 Description of the instrument

The instrument facilitates the constant and homogeneous recording of the phenomena subject to observation, thus simplifying the control of scientific observation.

This favors the achievement of systematicity, one of the fundamental values of science, in addition to improving the precision of observations. (Chowdhury, et al, 2019; Bandalos, 2018)

The choices among the many methods of collecting fisheries data depended on the variables measured, the origin and the resources available. The instrument to be used in this study corresponded to a format designed for the reading of slide slides, in which cytogenetic damage in the species Orechromis sp, treated with the compounds evaluated in this study, was analyzed.

4.2.3 Data sources.

The data were obtained from blood samples obtained from the fish found in the different fish tanks, with various treatments.

4.2.4 Population and sample

The population was made up of approximately 600 specimens of *Oreochromis sp* (red tilapia) from which a sample of 40 fish was taken, chosen in the shelters of the pools in the region of the Mesa Corregimiento of the Municipality of Valledupar.

5. RESULTS AND DISCUSSION

5.1 Presentation of the results

The results of this work were organized taking into account the stages of its elaboration, below, they are detailed in that order.

- The application of the Micronucleus test in the species *Orechromis sp* (red tilapia) was implemented as a pedagogical strategy where DNA, the environment and genotoxic agents were related
- Micronuclei present in fish erythrocytes formed from genotoxic agents were identified through the micronucleus test.
- When applying Pearson's correlation test, it was found that there is a positive correlation between the tested dose and the percentage of cytogenetic alterations, with a value of 0.94.

5.1.1 Results found in Paranitrophenol

It was expected that Paranitrophenol, in the concentrations tested, would induce damage at the level of the genetic material of the treated fish, which would allow determining the genotoxic risk to which some human populations are exposed.

The following are the results of the observation of micronuclei identified in the different groups of fish used to evaluate the effect of paranitrophenol in *Oreochromis sp.* The results obtained for each fish (4000 cells), and for each group of fish (20,000 cells/5 fish) are reported. The group of untreated fish (negative control) showed a total of 14/4000 cells with MN (Micronuclei), which is equivalent to 0.07%. Fish treated with tamoxifen showed 118/4000 micronuclei, which corresponds to 0.59%. In the case of fish treated with 10 **ng/ml** PNP (paranitrophenol), there was a total of 26/4000

micronuclei, which is equivalent to 0.13%, while fish treated with 20 ng/ml showed 32/4000 micronuclei (0.16%). Finally, the fish that received treatment of 40 **ng/ml** showed 72/4000 micronuclei (0.36 %). The results obtained allow us to infer that the evaluated concentrations of paranitrophenol in the treated fish present a dose-dependent response, in such a way that, the higher the concentration of the compound, the higher the percentage of genotoxic damage. When determining Pearson's correlation value, a value of 0.84 was observed, which corresponds to a positive correlation between the amount of paranitrophenol and the percentage of genotoxic damage.

5.1.2 Results found in malathion

Similarly, it was expected that malathion, at the concentrations tested, would induce damage at the level of the genetic material of the treated fish, allowing to determine the genotoxic risk to which some human populations are exposed.

The results of the observation of micronuclei identified in the different groups of fish used to evaluate the effect of malathion on *Oreochromis sp*. The results obtained for each fish (4000 cells), and for each group of fish (20,000 cells/5 fish) are reported.

In the case of fish treated with 10 **ng/ml** malathion, there was a total of 10/4000 micronuclei, which is equivalent to 0.05%, while fish treated with 20 **ng/ml** showed 14/4000 micronuclei (0.07%). Finally, the fish that received treatment of 40 **ng/ml** showed 26/4000 micronuclei (0.13%). The results obtained allow us to infer that the evaluated concentrations of malathion in the treated fish present a dose-dependent response, in such a way that, the higher the concentration of the compound, the higher the percentage of genotoxic damage. When determining the Pearson correlation value, a value of 0.92 was observed, which corresponds to a positive correlation between the amount of malathion and the percentage of genotoxic damage

5.2 Interdisciplinary Approaches

Different interdisciplinary approaches to education can have different connotations, as long as they are interested in the same area, but they could cover all scientific areas that channel their knowledge in a truly interrelated way, such as applying mathematical tools to biological issues or vice versa. However, it would be useful to consider whether the interdiscipline reaches different educational levels, with methodologies, strategies, modalities and forms of self-learning, creative self-generation of knowledge from critical reading, and from the active participation of students in experimentation, in the taking of problems from their own economic, productive, sociocultural, historical, institutional, family, religious contexts, political, everyday, geographical, and urban or rural; and even in the subsequent analysis and ethical reflection of the results. In both cases, analyzing not only local problems but also national and international ones, in comparative understanding.

It is convenient to report a reflection that it is not convenient for both levels of interdisciplinarity to be necessarily mixed, but, on the contrary, while in freedom and creativity the first two sublevels can be the necessary instruments to produce scientific knowledge. That third sublevel, often not avant-garde but traditional, of given interdiscipline, must follow certain scientific paradigms already accumulated, many of which are irrational and often prove the opposite.

5.3 Integration of Natural Sciences and Mathematics in Teaching

Integration of Natural Sciences and Mathematics in Teaching: The changes that are coming, not only in the country's education, but worldwide, also require changes in teaching strategies to prepare students with the skills required by the globalized world in which we are going to live. This is how the concept of "thorough integrals" is becoming more and more indispensable both for Physics, Chemistry and Biology laboratories and for mathematical knowledge from the first semesters and not as isolated spaces until today. In this sense, when implementing this work in the subject of Cytogenetics, students must attend for 8 hours; however, the collection and analysis of the data is carried out in a Chemistry laboratory, which represents about 3 additional hours of advice to count the number of cell divisions and establish the type of chromosomal aberration obtained in the tested population.

6. RECOMMENDATIONS FOR TEACHING

A change is required in the teaching of the area of natural sciences so that it takes conceptual elements from other disciplines, seeking a real integration in the student. This would be achieved through the implementation of the PNEA, taking into account that the student must be provided with significant

experiences that are related to their environment, daily life and environment, promoting and developing in the student creativity, innovation, teamwork, interdisciplinarity, interculturality, love for science, a fundamental element for the development of critical and conscious human beings. Aspects that traditional teaching loses. The PNEA can be developed in each of the experimental activities as an integrating model and not just an end in itself, thus achieving a global development of the student. (Alvarez et al, 2021)

Mathematics is a tool that has allowed us to better understand and solve scientific problems. The approach of mathematical models has facilitated the approach to much more feasible and plausible solutions. In no way can mathematics be considered as a finished structure; on the contrary, it allows us to interact constantly in the modeling of real situations, thus establishing a process of mutuality for the benefit of all parties. Mathematics is not the absolute truth; therefore, the teaching of a mathematical model is meaningless if a permanent dialogue with the real problem is not established. The instrumental character conferred by its cultural origin has limited it in that non-mathematical culture has not been able to give it a legitimate space of meaning and interpretation. It is never too late to understand that the development of mathematical thought has its roots in individual and collective interactions with concrete and abstract realities that are and always will be scientific, artistic, philosophical, political, economic-productive and religious.

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