Principles of problem-based learning for training and professional practice in ecotoxicology

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Highlights

- PBL is an efficient strategy to train future ecotoxicology professionals.
- A case study using fish biomarkers for the development of this strategy is discussed.
- Simple statistical analyses were carried out to infer differences among fish sampling points.
- Teaching ecotoxicology through PBL allowed students to develop several skills.
- Further intervention studies on ecotoxicology teaching through PBL are required.

Abstract

Problem-based learning (PBL) is a protagonist of constructivism widely used successfully in higher education. PBL is a learner-centered instructional and curricular approach that can use real problems for the development of the teaching and learning process. On the other hand, the complexity of knowledge of Ecotoxicology, as well as the importance of this field for Environmental Health and society demand reflections and proposals for the training of professionals who work in this field. Therefore, in accordance with the principles of PBL, this strategy can effectively contribute to the training and professional practice of ecotoxicologists. We report herein the importance of the principles of PBL for the training of ecotoxicologists, including the discussion of a case study using fish biomarkers for the development of this teaching strategy. Teaching ecotoxicology through PBL principles allowed students to develop several advantages for Ecotoxicology training through the discussion of an authentic, real life problem that engaged them in its discussion, functioning as a stimulus for learning. The students were able to develop laboratory skills, group work competences, understand basic concepts and fundamentals of Ecotoxicology and become empowered regarding skills and competences for future practice.

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1. Background

This article discusses how problem-based learning (PBL) principles can contribute to the initial training and professional practice of ecotoxicologists. PBL was systematized as a teaching strategy and curriculum organization at McMaster University (Canada) in the 1960s and is currently applied as a teaching and curriculum organization strategy in different countries and many higher education courses worldwide (De Pinho et al., 2015). It is noteworthy that the last decade has seen an increased focus on inquiry-based instructional approaches to quell student attrition in science, technology, engineering, and mathematics (STEM) disciplines (Beier et al., 2019). Thus, PBL has introduced new ways to teach STEM-based subjects to students. In this regard, we defend the use of PBL as an efficient strategy to train future professionals, under a perspective in which students face the complexity of real life problems, and the need to integrate knowledge and face situations closely related to the real world (DiCarlo, 2006; Jansson et al., 2015).

PBL promotes curricular integration and places students at the center of the educational process, giving them greater autonomy and responsibility regarding their own learning processes (Neville and Norman, 2007). In PBL, students can engage in real-world problems in an attempt to find solutions. In these scenarios, problems, tutors and small groups are essential PBL components (Dolmans and Schmidt, 2006). The problem is crucial and is the starting point for the learning process. Tasks are built with pre-established objectives and, whenever possible, taken from student realities and from professional student disciplines. These teaching and curricular organization potentiate critical sense, creativity and the ability to lead work teams and autonomy from students (Wood, 2003). On the other hand, in a complex and fast-changing world, ecotoxicology and ecological sciences are essential to address intricate environmental questions and contribute to the understanding and resolution of local, regional, and global environmental problems (Lewinsohn et al., 2015). Therefore, PBL can be seen as an important contribution to the professional development of ecotoxicologists.

Ecotoxicology as a field of knowledge and ecotoxicologists, as actors responsible for its development can play an important role in relation to the 17 United Nations Sustainable Development Goals. For instance, with regard to climate action, clear water and sanitation, sustainable cities and communities, life below water, industry, innovation and infrastructure, and quality education (Bhore, 2016). In this scenario, ecotoxicology represents a scientific field that investigates the impact of chemical contaminants on the environment at the molecular, physiological, individual and ecological levels, encompassing both laboratory and field methods (Beketov and Liess, 2012). This field comprises the integration of ecology and toxicology in understanding and predicting the effects of chemicals on natural communities under realistic exposure conditions (Chapman, 2002). For example, the control of toxic agents is carried out by the determination of specific substances, for which numerical emission and concentration standards have been established (Coimbra et al., 2018; Lopes et al., 2018).

The individual and quantitative evaluation of toxic compounds in the aquatic environment is, now more than ever, an important and current issue (Aljerf and Al Masri, 2018). However, these analyses display certain limitations, such as 1) the inability to identify all toxic substances in effluents presenting a complex chemical nature; 2) the fact that the toxic effects that a compound identified by chemical analyses may present to the biota may not be known; 3) the fact that a chemical compound may interact in a synergistic, antagonist or additive manner with other compounds, whose toxic effects cannot be evaluated solely by the detection of a certain substance in water; 4) toxic effects can be altered by certain water characteristics, such as salinity, pH, temperature, oxygenation and hardness and 5) toxic agent bioavailability is frequently difficult to determine (Bolis et al., 2001). Therefore, ecotoxicology knowledge is useful in indicating the magnitude and likelihood of adverse effects caused by chemical compounds released into the environment. Moreover, ecotoxicological methods may be applied in the control and evaluation of environmental impacts, in determining the efficiency of the technical procedures adopted to reduce these impacts and in generating information for government agencies and those in charge of environmental pollution control programs. Reporting of ecotoxicology studies must be appropriately utilized, based on their reliability and relevance, for environmental protection (Hanson et al., 2017).

Research in multiple areas, including Ecotoxicology, is, however, required to further advance knowledge and reduce knowledge gaps concerning pollution and its potential health effects, contributions to the global disease burden and economic consequences (Landrigan et al., 2018). In this context, we agree with Lewinsohn et al. (2015) that a significant education challenge is the development of an education system capable of presenting the relevance and the solidity of scientific concepts to students through connection and “confrontation” with real-world problems, concentrating on practical applications in the real-world contexts of future professionals and/or researchers. Therefore, a new form of training regarding professionals that will someday act in the Ecotoxicology area, as well as in other areas of knowledge, becomes necessary (Hulla Janis et al., 2015), and PBL can be seen as a potential strategy to address this issue.

Martin Scheringer (2017) warns that ecotoxicology and environmental chemistry are often seen in universities as traditional and outdated fields (Scheringer, 2017). Therefore, investment and research in ecotoxicology teaching is paramount for this subject not to be presented and perceived as a simple list of routine tasks that must be performed (Beketov and Liess, 2012). Moreover, in addition to the importance of combining basic research and applied (practical) research, it is crucial to highlight that ecotoxicology investigates a complex set of socially relevant issues and impacts, in which the environmental and health impacts of pollution are no less important than other environmental impacts (Scheringer, 2017).

A simple applicable approach to ecotoxicology through PBL is through hands-on experience, such as environmental health assessments through the use of biomarkers and biocindicators. In addition, it is also important for students to explore issues surrounding their local environment, which makes them more aware of potential problems or issues directly relatable to their daily lives (Kamaludin et al., 2018). Driven by these considerations, in the next section we discuss a case study describing a set of activities for graduate students in ecotoxicology training based on the use of PBL and using fish biomarker assessments is presented below.

2. Real scenario exploration

In Brazil, reservoirs have now been constructed on almost all the country’s hydrographic basins, for water supply, irrigation and mainly, electricity generation, directly impacting many local populations. However, despite their benefits, they also lead to serious and irreversible environmental alterations, which may be assessed through the use of biocindicators (Agostinho et al., 2008). Aquatic organisms, fish in particular, are recognized as excellent biocindicators regarding environmental alterations, as they are sensitive to impacts and can give early warning signs about potential deleterious effects of several environmental modifications in aquatic ecosystems (Espino, 2000). Their biochemical (cellular, molecu-
lar, organ and systemic) responses to pollution are termed biomarkers, and, when assessed together, display the potential to assist in water quality evaluations and environmental risk and environmental health and safety assessments (van der Oost et al., 2003).

The tucunaré (Cichla ocellaris) is a territorial cichlid that inhabits mostly high-temperature lentic transparent waters, frequently presenting as the dominant diurnal piscivore (Winemiller, 2001). This species was introduced as game at the Juturnaíba Dam (22°36′43.6″S 42°16′29.25″W), the only fresh water source supplying the entire Lake District, comprising eight municipalities and almost 600,000 inhabitants, located in the state of Rio de Janeiro – Brazil (De Morais et al., 2016).

As this is a top food chain predator and highly consumed by humans, this species displays both environmental and economic importance. The proposal herein was to use the tucunaré as a bioindicator to evaluate the environmental health of the Juturnaíba Dam, by developing a problem to be solved by professors (n = 2), master’s and doctoral students (n = 5) attending the Postgraduate Program in Public Health and Environment at the National School of Public Health Sérgio Arouca (ENSP), belonging to the Oswaldo Cruz Foundation (Fiocruz). As mentioned previously, the integration of laboratory work and PBL-based activities favors laboratory work contextualization concerning the subject matter and promotes Science-Technology-Society-Environment relationships (Janson et al., 2015; Llorens-Molina, 2010).

3. Methods

This study does not aim to detail laboratory analyses and procedures. Briefly, all biochemical analyses were carried out at the Oswaldo Cruz Foundation laboratories, through several standardized and published experimental protocols (Dos Santos et al., 2016; Lopes et al., 2018). Experimental procedures were carried out according to the ethical principles of animal experimentation elaborated by the Brazilian College for Animal Experimentation (COBEA), in agreement with the uniform requirements for manuscript submissions to biomedical journals.

3.1. Fish sampling

Twenty-one specimens, 11 individuals from a defined reference area (C) and 10 from a defined impacted area (E) were captured by artisanal fishermen at the Juturnaíba Dam and transported to the banks. This number of samples is considered enough for adequate statistical assessments and may be extrapolated as representative of a population in a certain condition (Ontario’s Ministry of Environment, 2013). The sampling areas were determined by the presence of an effluent discharge point from a water treatment plant (WTP) on the reservoir margins, where upstream of the WTP effluent emission was considered the reference area and downstream, considered the impacted region. Sampling consisted of two boats leaving for both areas at 6:00 AM. Each team consisted of a pilot and an artisanal fisher with complete equipment for artificial bait fishing. Each boat was equipped with an aerated life support tank for the fish, which taken ashore alive. The entire capture strategy was organized by the students in formation, based on the real scenario and the need for a biomonitoring program using the tucunaré as a bioindicator. All pedagogical activity respected the working fundamentals of Problem Based Learning.

3.2. Fish anesthesia and biometric assessments

Prior to blood sampling, the animals were anesthetized with eugenol in potable water at a final concentration of 30 μL L⁻¹, from a 50% eugenol stock solution (v/v) in hydrated ethanol. Fish were considered anesthetized when their position in the water became random, with the prevalence of the dorsal position and no body response to external stimuli (Rocha-Santos et al., 2018). Once anesthetized, the fish were rapidly removed from water, weighed, measured (furlar length – Fig. 1) and photographed.

3.3. Fish blood collection, euthanasia and tissue collection

Blood collection for biochemical analyses was performed with syringes rinsed with sodium heparin for pharmaceutical use. A 0.3 mm gauge needle was used to reduce any lesions caused by the sampling, and the blood was drawn through the caudal vein. Blood samples were maintained at 5 °C for a maximum of 24 h prior to the biochemical determinations. After blood collection, the animals were euthanized by marrow sectioning. The liver, spleen, second branchial arch, stomach and a sample of muscle tissue were collected for future analysis from each animal (Carmo et al., 2019; Ramesh et al., 2018).

3.4. Haematological parameter determinations

Fish haematological parameters are routinely applied as biomarkers of effect concerning aquatic contamination (Lermen et al., 2004). For example, the leakage of specific enzymes (e.g. transaminases) into the bloodstream may be indicative of the disruption of cellular membranes in certain organs, and other parameters such as hematocrit, hemoglobin, catalase activity and glucose levels have been reported as being sensitive to certain types of pollutants (Dos Santos et al., 2016; van der Oost et al., 2003). The following biochemical parameters were determined in tucunaré blood samples:

a) δ-aminolevulinic acid dehydratase (ALA-D): a key enzyme in the heme biosynthesis pathway, and a selective and fast-responding biomarker to lead exposure and bioavailability (Fernández et al., 2015).

b) Catalase (CAT): a hematin-containing enzyme that facilitates the removal of hydrogen peroxide (H₂O₂) and metabolizes this reactive oxygen species to molecular oxygen (O₂) and water (El-Desoky et al., 2016; Unfried et al., 2007). CAT activities in erythrocytes may be a more appropriate marker for oxidant exposures in vertebrates (oxidative stress parameter) (Zamocky et al., 2008).

c) Hematocrit (HT) and hemoglobin (Hb) levels: the former determines the volume of red blood cells compared to the total blood volume (red blood cells and plasma) and the latter is the protein contained in red blood cells responsible for oxygen delivery to tissues (Billett, 1990).

d) Glucose levels: Blood glucose levels are indicative of amount of glucose present in blood at a given time and routinely applied as a stress biomarker (Cox and Nelson, 2013).

Fig. 1. Tucunaré (Cichla ocellaris) specimen caught at the Juturnaíba Dam during the PBL study.
e) Serum transaminases (aspartate amino transferase – AST and alanine aminotransferase ALT): Increased enzymatic transaminase activity in fish blood may indicate the involvement of membranes or cells in systems such as the liver (Coz-Rakovac et al., 2008; Kramer and Hoffmann, 1997).

3.5. Statistical analyses

Data normality was assessed by the Shapiro-Wilk normality test and differences between reference and impacted groups were assessed either by the unpaired t-test when normally distributed, or by the Mann-Whitney test, when a non-normal distribution was presented. Statistical significance was set at p < 0.05. All statistics were carried out using the Prism 5.0® software.

4. Results and discussion

Tucunaré biometric and haemotological parameters are displayed in Table 1. All statistical analyses concerning tucunaré biometric and haemotological parameters were carried out by the students. A normal data distribution for weight and length data and a non-normal distribution for the haemotological parameters were observed. Thus, differences for the former were assessed by the unpaired t-test and for the latter, by the Mann-Whitney test. No significant differences (p < 0.05) between the animals were noted for biometric parameters, indicating that the animals, in general, displayed the same feed conversion rate and can be grouped together for assessments. Among the assessed biochemical parameters, only ALT was significantly different (p < 0.05) between groups. Taken together, the results did not reveal any indications of poor health for the tucunaré specimens caught at the Juturnaíba Dam.

Fig. 2 presents photomicrographs of gill images fixed in formalin and observed under a dark field microscope. The two lamellae of the second branchial arch of all fish were examined. The branchial arch was observed dry under a 4× objective. Some anomalies, including both thinned and engorged lamellae tips, and the presence of lamellae, were observed in animals 5, 6 and 7. It is important to emphasize the simplicity of this morphological evaluation, which has not yet been formally described in the literature. The results presented herein, however, may formalize the use of this method in environmental impact assessment programs, using fish species as environmental bioindicators.

Several educational implications can be derived from the proposal presented herein. To illustrate this, an overview of the potential of PBL principles for Ecotoxicology Training is shown in Fig. 3.

Obviously, other bioindicator species, biomarkers (of effect, exposure, or susceptibility), or other real-life scenario could be applied as case studies. In most cases, the advantages of using PBL would include all the aspects displayed in Fig. 3, such as:i) the development of laboratory skills; ii) the competence to work in groups; iii) the understanding of basic concepts and fundamentals of Ecotoxicology; and iv) the ability to empower learners regarding skills for future professional practice (for example, in designing projects). These are essential qualities in a period of rapid knowledge information evolution and dissemination. In addition, this PBL approach concerning ecotoxicological applies simple techniques, which may be used by students in almost any real-world scenario worldwide, including in developing countries or in areas with no funding access.

It is noteworthy that PBL “is an instructional (and curricular) learner-centered approach that empowers learners to conduct

<table>
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<th>Group</th>
<th>Sample Code</th>
<th>Length (cm)</th>
<th>Weight (g)</th>
<th>HT</th>
<th>Hb</th>
<th>Glucose</th>
<th>ALAD</th>
<th>CAT</th>
<th>ALT</th>
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(4) – HT = Hematocrit (%); Hb = Hemoglobin (g dL⁻¹); Glucose = plasma glucose (mg dL⁻¹); ALAD = δ-aminolevulinic acid dehydratase (U); CAT = catalase (U); ALT = alanine transaminase (U); AST = aspartate amino transaminase (U). * indicates a statistically significant difference between the reference and impacted groups.
research, integrate theory and practice, and apply knowledge and skills to develop a viable solution to a defined problem” (Savery, 2006). The learning process starts with solving problems, which are organized in activities, in processes termed the PBL tutorial or PBL cycle (Hmelo-Silver, 2004; Hung, 2009). In this cycle, students work in small collaborative groups and the teacher acts as a facilitator or guide for the student learning process (Hmelo-Silver, 2004). Thus, PBL can be characterized by i) learning in small groups; ii) a teacher facilitates group learning; iii) learning is carried out by means of problems that are first discussed by the group; and iv) learning is carried out by an instructional approach in which the problem is the stimulus for learning (Dolmans and Gijbels, 2013). All these preconditions were respected in the performed activities, by the five students and two teachers/tutors during the described activities.

PBL increased the possibility of curricular integration and became closer to more realistic approaches to environmental problems, thus increasing student motivation and engaging them in problem solving and project development (LaForce et al., 2017; McGibbon and Van Belle, 2015). However, collaboration between educator and researchers is critical for leveraging change processes and delivery of educational interventions, including those generally related to sustainable development (Sacchetti and Calliera, 2017); as well as change in the curricula or methods employed to teach in undergraduate, postgraduate and other levels of education. In this sense, greater articulation, dialogue and cooperative work between departments and professionals through technical and pedagogical training can become essential links for changes in educational institutions (Dalati and Al Hamwi, 2016).

The activity described herein was performed as a case study illustrating the application of PBL fundamentals to a group of five postgraduate students in ecotoxicology (seemingly the first article of this type to be indexed in the Web of Science). However, for larger groups of students, cooperative efforts to deploy hybrid curricula or hybrid-PBL (incorporating PBL principles, Project Learning or, Investigative Case Based Learning) can lead to the development of interesting models to be applied in courses for ecotoxicology training. In other words, Hybrid-PBL (H-PBL) can be adopted as a good alternative to implement PBL principles to ensure a “non-exclusively traditional” or teacher-centered learning process for ecotoxicology learners. As an example, Carrio and colleagues implemented an H-PBL model in the biology undergraduate curriculum where 20% of the teaching time was devoted to PBL activities (Carrio et al., 2016). This same study, and others, point out that H-PBL is better than the traditional lecture-based learning method at improving basic student knowledge for long-term knowledge acquisition and problem-solving skills, and that students displaying a significant preference for hybrid-PBL (Jimenez-Saiz and Rosace, 2019; Lian and He, 2013).

The scenarios or problem situations investigated in PBL involve students making decisions. Students become “stakeholders” in problem solving. Herein, the real scenario or problem presented to the students was the need to design and execute a biomonitoring
project at Juturnaíba Dam. As described previously, teachers act as tutors throughout the process described in the PBL cycle. Nevertheless, lectures are not totally excluded from the PBL curriculum, and can be used as complements whenever necessary. At the same time, this type of curriculum gives the trainee more potential for promoting lifelong learning skills and “deep learning”, including conceptual understanding (Dolmans et al., 2016). Other studies, especially systematic reviews and meta-analysis, offer evidence that PBL contributes to positive effects after graduation, especially in the social and cognitive dimensions (Dochy et al., 2003; Koh et al., 2008).

Group processes include all intrapersonal and interpersonal actions by which the group transforms its resources into a product (Chiriac, 2008). Small groups in PBL can be considered from the perspective of a complex adaptive system, in which students interact with each other according to a few rules in an environment from which learning and understanding will emerge (Mennin, 2007). Learning assessment in PBL is performed in this formative context, which evaluates the learning and discovery process by which students used to achieve their goals and solve problems or apply content knowledge to a practical real problem (Lopes et al., 2011). These characteristics were noted by the teachers (tutors) in the development of the set of activities performed by the students, such as the planning of tucunaré capture, the study of the choice of biomarkers that may be applied in ecotoxicological assessments, the survey of the performed laboratory methodologies, the laboratory analyses themselves, the construction of charts and tables for the result analysis, and the preparation of reports concerning the performed research.

5. Final considerations

Without the possibility and intention of exhausting such a complex subject, such as curricula structuring and the construction of teaching proposals for an integrated and complex field of knowledge as ecotoxicology, this article seeks to shed light on these processes and contribute to training in this area through the use of PBL. PBL is, thus, an alternative model to the traditional model, highlighting, among other aspects: i) the insertion of students at the center of the educational process; ii) the organization of the curriculum or subjects to be studied around real and holistic problems, allowing students to learn in a meaningful and articulate manner and; iii) the generation of a learning environment in which teachers guide student thinking and research, facilitating deep levels of understanding of each problem presented. In this way, learners tend to gradually develop a set of skills and attitudes that are paramount towards excellence in the development of ecotoxicological studies.

This study presents certain limitations, as it is characterized as a case study involving a small group of students in ecotoxicology training through PBL. Concerning future perspectives, intervention studies of ecotoxicology teaching through PBL can and should be performed, enabling data collection and the analysis of aspects involving student motivation, conceptual learning and the development of metacognitive skills by learners, as well as research with researchers, professors, and directors of universities and other educational institutes, in order to evaluate the real possibility of change in the teaching models adopted by these institutions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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