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Problem-Based Learning on Cell Biology and Ecophysiology using integrated laboratory and computational activities

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Abstract
Since all the known biological systems require water for their basic biochemical processes, one can find several osmoregulation mechanisms on living organisms for adaptation to related environmental challenges. Osmosis is a cellular mechanism of water movement across membranes which is known to be present throughout the tree of life and occurs by either diffusion across the membrane bilayer or by a faster movement mediated by transmembrane channel proteins, called aquaporins. The expression of aquaporins is regulated at, the cellular level, by environment conditions such as hydric stress, therefore allowing the adaptation of organisms to increase salinity in soils, water deprivation and increase beverage intake.

Osmosis and diffusion concepts have been described to be difficult to learn, so, in order to promote meaningful learning, we used a problem-based learning approach that integrates a laboratory activity and a computer simulation model of osmosis and a two phase conceptual mapping.

We observed that high school students developed adequate laboratory skills and were able to communicate their results as text and using scientific drawings; and the learning environment was adequate. Therefore we presented a successful implementation case of integrated PBL, in a public Portuguese school, that may constitute an example to facilitate the implementation of active inquiry strategies by other teachers, as well as the basis for future research.

Keywords Problem-Based Learning; experimental activities; computer simulation; osmosis.

1. Introduction

Water is a condition *sine qua non* of life, however 97.5% of the total Earth's water content is found in the oceans and global warming and the consequent increase in sea level and soil salinization will diminish the productivity of farmlands throughout the world (Ivits et al., 2013).

At the cellular level, the influx (out flux) of substances into (out of) a cell or an organelle can occur either: (i) according to physical and chemical properties by a passive process from an area of high concentration to an area of low concentration or (ii) by an active process, consuming energy (ATP molecules), occurring against the concentration gradient and involving specific mediators on the cell membrane. The mechanism of water movement across membranes, from the side with lower solute concentration to the side with higher osmolality, is called osmosis and it is known to be present throughout the tree of life (Verkman, 2011), since this cellular mechanism presents some evaluative advantages such as not requiring consumption of any ATP molecule. This transmembrane movement occurs by either diffusion across the membrane bilayer or by a faster movement mediated by transmembrane channel proteins, called aquaporins - AQPs - and were the focus of a Nobel Prize (Agre, 2003).

Cells in hypotonic environment would increase in volume and evenly burst, hence the osmoregulation at the cellular level is an important process that is known to be mediated by vacuoles. Hence this osmolality problem is solved by living organisms by various mechanisms that continuously and actively pump ions out. Plant, algae, fungi and some bacterial cells have an additional barrier - the cell wall - that avoids the variation on total cell volume and the burst of the cells by water influx. However, turgidity is a necessary condition for plants to maintain their firmness or turgor.

It has been described the expression of AQPs in all organisms in their cellular membranes (Ishibashi et al., 2011), with the main role of water transportation, which constitute an evidence of evolution by adaptation of organisms to the environmental challenges related to water-based life. This cellular membrane permeability is selective, since water (H_2O)

crosses through the membranes, while the hydronium ion (H_3O^+) does not permeate these proteins, so this distinction is essential to life (Agre, 2003).

A limiting factor for plant growth and development is the hydric stress in hypertonic environments, such as due to water deprivation and increased soil salinity, that if continued and/or at high levels will cause plants to wilt and eventually shrivel and die. Plants have evolved several biochemical and molecular mechanisms to cope with these types of abiotic stress, such AQPs expression, therefore one can find some species and cultivars with higher drought and salt tolerance.

Recently, UNESCO created the Centre for Membrane Science and Technology, that is responsible for the development of desalination processes based on reverse osmosis, during which seawater is forced through a semipermeable membrane that separates salt from water, so this substantiate the importance of the study of transmembrane transport.

This theme constitutes an excellent example for students to learn about the existence of interactions between Science, Technology, Society and Environment (STSE education) since the development of the technological applications of reverse osmosis can be used at a large-scale to produce drinkable water from sea water, with potential economic and life expectancy benefits in several developing countries. Socio-Scientific Issues are also included such as the development of transgenic overexpressing AQPs plants that could be used in agriculture to increase yields and the development of drugs that modulate AQP expression and function can be applicable to medicine.

While PBL exists in a variety of forms, depending on the discipline and the goals of the curriculum, it tends to include features such as learner autonomy, active learning, cooperation and collaboration, authentic activities and reflection and transfer (Ertmer & Simons, 2006). Osmosis and diffusion concepts have been described to be difficult to learn, so, in order to promote meaningful learning, we used a problem-based learning approach that integrates a two phase conceptual mapping and experimental activities, such as a laboratory activity, testing the effects of osmolarity in plants and a computer simulation model of osmosis (Kottonau, 2011; Lindgren et al., 2009). The computer simulation was suggested in order promote inquiry learning since it allows students to specify a few parameters (e.g. concentration inside and outside the virtual membrane) and then observe the outcome (Lindgren & Schwartz, 2009).

Learning initiation occured by introducing a problematic situation that included a core question, consisting in a conceptual change eliciting question, designed to trigger learning. Other essential questions of different types were also included, such as conceptual change and motivation types (Yip, 2004) and the complementary experimental activities were designed to facilitate learning by developing students observational and laboratory skills.

2. Materials and methods

2.1 Participants

Study participants were students attending the tenth grade at a public school (high school, with age from 14 to 16 years, and 15 years as median age) that willing to participate in the study had the corresponding informed consent signed by the parent/person responsible for education (n = 18). The author participated in the instruction by teaching the corresponding curricular unit to all the students at a Portuguese public school (Oporto city, Portugal).

2.2 Educational intervention

The results presented were obtained during a one-week period of an action research project on PBL, during the academic year 2012/2013.

The PBL methodology used included an ill-structured problem with adequate guidance by the author/tutor according with age and unfamiliarity with PBL of the students. Hence the author/tutor promoted the discussion and the questioning by the students using an electronic presentation in each lab-class (≤ 14 students). The open-ended question proposed to students for this class was ”Is it beneficial for the organisms that life is based on the existence of water?”, and the students discussed, in small groups (≤ 4 students), and proposed new questions and possible solutions to this problem.

The theme is included in the discipline Biology and Geology in the curricular unit on the diversity of mechanisms for obtaining nutrients used by different organisms.

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On the first day the duration of the activities was 135 minutes and then 45 minutes at the end of the week for discussion and elaboration of the post-conceptual map.

2.3 Experimental activities

2.3.1 Laboratorial experimental activities

In this study we used *Pelargonium x hortorum,* red flowers cultivar, native of South Africa, that is commonly used as an ornamental plant in Portugal.

The experimental setup consisted in three groups of plants maintained in mineral soil-containing vases watered, every other day, during one week, with solutions with different salinity, containing different concentration of minerals (Table 1).

Table 1. Culture conditions for maintenance of each group of plants *Pelargonium x hortorum*.

Group A	Group B	Group C
mineral soil + mineral H_2O (H_2O total mineralisation: 47.7mg/L)	mineral soil + 12% NaCl (in deionized H_2O)	mineral soil + deionized H_2O

Note: Deionized water was obtained by the students using the equipment available in the physics-chemistry lab of the school; as well as the 12% NaCl solution (using NaCl, Sigma). Commercially available water was used for plants belonging to group A.

Students observed the macroscopic morphology of plants of groups A and B and were asked to predict the morphology of the group C of plants.

For the microscopic observations, we used petals of the flowers of this plant since they present hydrophilic pigments in vacuoles that are responsible for the red colour of the petals. So, students, in small groups (≤4 students), prepared samples by peeling a piece of the superior epidermis of petals of plants of group A and placed two of these samples in two conditions, in slides containing one drop of either 12% NaCl (sample A1) or mineral H_2O; then observed both under the microscope and illustrated a few representative cells observed in each slide. The media of sample A1 was then carefully replaced by deionized H_2O and observed under the optical microscope and illustrated (sample A2).

2.3.2 Computational experimental activity

In the second part of the lab report students were asked to answer the question "What happens to water molecules when cells are in an isotonic medium?", so in order to observe what happens at the cellular level in plant cells under an isotonic environment, students designed and performed a virtual experiment using the computational simulator of osmosis available online - "NetLogo Osmosis Simulator" (Kottonau, 2011).

2. 4 Instruments

A heurist diagram including the laboratorial procedure was provided to the students and they were asked to complete and present it, at the end of the class, as the lab report to be evaluated by the teacher/author.

Students, in small groups, were asked to elaborate a conceptual map on transmembrane transport. Then, after referring the concept of aquaporins, a conceptual map, with a defined skeleton structure, was provided to the students and they were asked to complete it with the missing concepts and liaison terms (Fig. 1).

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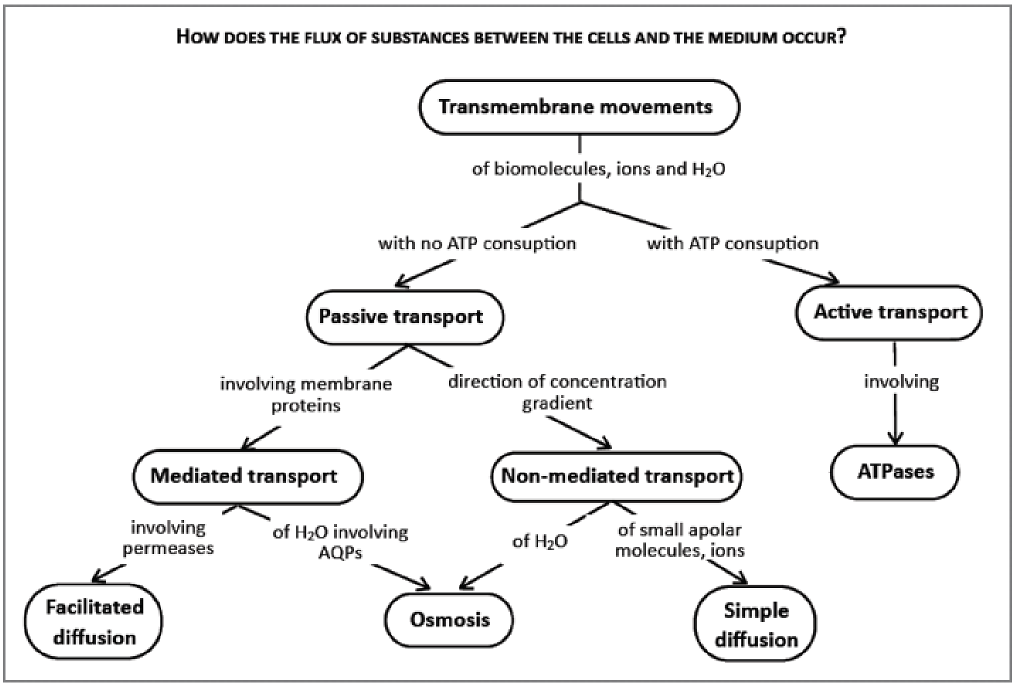

Figure 1. Portion of the conceptual map on transmembrane movements (referring only to biomolecules, ions and H_2O) completed by high school students.

The problematic situation, with the central question "Is it beneficial for the organisms that life is based on the existence of water?", as well as the corresponding PBL worksheet, in which students were asked to complete the list of facts and questions related to the topic and to define an experimental question, were provided to students as hands-outs and were discussed in small group in class.

The lab report based on the heurist diagram that students were asked to complete, and that also included the request to illustrate their observations under the microscope, using the adequate magnification, including the arrangement of a few number of cells within the tissue; and to answer some open-ended questions as well as to write a small text on Science, Technology, Society and Environment interactions about osmosis.

To assess the impact of our strategy on learning and long-term retention and ability to apply knowledge in an integrated way, students' scores obtained in specific questions on the exam at the end of the unit were analyzed.

At the end of the class, students were asked to respond to a questionnaire in order to evaluate their perceptions about the PBL environment in class, regarding some dimensions, according to Senocak (2009), such as: teacher support (the extent to which the teacher acts a facilitator or metacognitive coach and supports several of the goals of PBL), student interaction and collaboration (the extent to which problems act as stimulus and PBL starts with an ill-structured problem to be solved) and the quality of the problem (extent to which problems act as stimulus and PBL starts with an ill-structured problem to be solved).
Students were asked to respond a small questionnaire about their self-perceptions of the contribute of each activity in their learning.

3. Results and discussion

Conceptual mapping has been describing as an useful strategy for learning and assessment (Schönborn & Anderson, 2008), so we used it to assess the preconceptions of students about osmosis before the last round of instruction: 70% of students classified osmosis as a non-mediated type of transport and 70% of the students classified it as a passive type of transport. At the end of the class, after discussion about AQPs, all the students were able to correctly complete the conceptual map in Figure 2, corresponding to meaningful relationships between concepts.
Since non-integrated classes have been described not bridge the gap between what students learn during PBL classes and the adequate laboratory skills, we used an integrated PBL approach (Azer et al., 2013) by designing a problematic situation, or problem, and scaffold strategies that promote the development of the desired capabilities.
Upon discussion of the problem in small groups, 83% of the students were able to define an adequate experimental question as "What are the effects of increasing salinity at the cellular level?", and "What are the differences at the cellular levels of plants maintained in normal soils and in soils with higher salinity?".
All the groups of students completed correctly the list of facts and proposed several adequate and interesting questions, of high order, some of which were selected for further

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study, such as: “In what way is water essential to life?”, “What happens at the cellular level responsible for human death by over digestion of water?”, “Why is the distribution of water in the planet non uniform?” and “Is it possible that drinkable water ends in the near future?”.

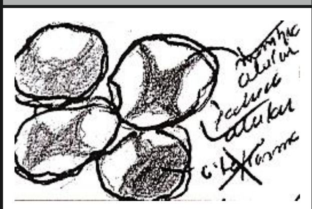
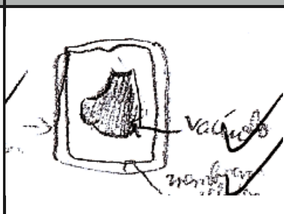
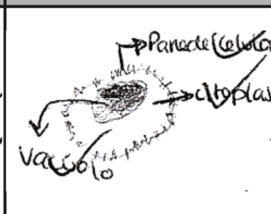
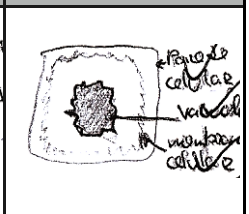
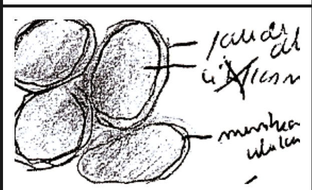
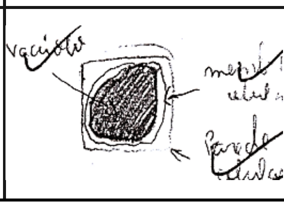
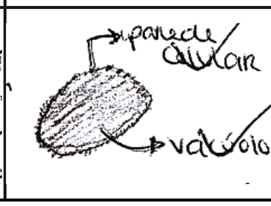
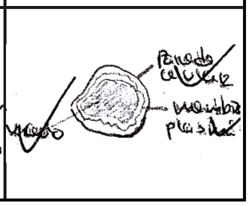
At the end of the class, all the students were able to propose a possible solution to the central question, referring to the benefits and the challenges of water to known living organisms.

More than 80% of the students were able to predict that plants of group C would be similar to the plants of group A and that their cells should be turgid, and they considered that observation alone, at the macroscopic level, of this process is not enough, and that further observation at microscopic level are necessary (Tomažič & Vidic, 2011).

By asking students to make scientific (anatomic) drawings it is possible to promote the development of several skills including the abilities to interpret complex information and to distinguish among similar structures, as well as communication skills in a visual form.

Students developed the adequate laboratory skills since they were able to select the adequate magnification and field of view for drawing and most students were able to illustrate some details of individual cells and represent form, proportion, and spatial relationships accurately (Table 2).

Table 2. Drawings by students of microscopic observations of *Pelargonium sp.* (petals of the flowers).

	Student 1	Student 2	Student 3	Student 4
A1				
A2				

Note: Drawing were made upon observation under microscope using a 40x objective. Cells of sample A1 were maintained in slide media containing 12% NaCl, and this media was then replaced by deionized H_2O (condition A2).

By analyzing the students' drawings (Table 2) we were able to observe some misconceptions, such as 35% of students that do not distinguished the vacuole from the cytoplasm (e.g. student 1 in table 2). In the second session we used these misconceptions as an opportunity to discuss with students the plant cell structure and functions of the vacuole and tonoplast, as suggested by others (DiCarlo, 2006), and asked students to redraw and correct their legends.

Upon the replacement of slide media containing 12% NaCl (sample A1, Table 2) by deionized H_2O (sample A2, Table 2), all students were able to observe an increase in the vacuolar volume in most cells, and elaborated the conclusion that the vacuole and cytoplasm shrinkage observed is a reversible process, under these conditions.

More than 80% of students were able to quantify the vacuole volume in both samples, completing correctly the graph provided in the corresponding question of the lab report.

Osmosis and diffusion concepts have been described to be difficult to learn, may be due to the fact that these processes involve invisible particles and constitute abstract ideas (Fisher et al., 2011). However, all the groups of students were able to perform the experiment using the computer simulation by adjusting the same concentration inside and outside the virtual membrane and all students concluded that water molecules are constantly going inside and outside the cell, as a dynamic equilibrium, maintaining the cell and vacuole volume.

The students' laboratory skills were assessed by the tutor/author, by observing during the class the complying with safety rules and laboratory manuals, communication skills and productive working skills, and the results were: Excellent for 32% of students, Good for 47%, and Satisfactory for 21% of the students.

50% of the students were able to write a small text, as asked in the final question of the lab report, about osmosis referring the interactions science-technology-society-environment in a future scenario of increase of sea level by climatic changes.

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The lab activity was successful since we observed positive classification of the lab report of all students, and 37% had an Excellent grade (corresponding to a classification of 85% or higher) and 26% were classified with Good (≥ 75% and < 85%) and 37% with Average (< 75% and ≥ 50%).

Regarding long-term retention, 78% of the students were successful (≥50% score) in recalling and applying knowledge, while 22% of the students obtained an insufficient score in the questions about osmosis in the exam at the end of the unit.

The analysis of the responses to the questionnaire that evaluates the perceptions of the students about the PBL process shows that the learning environment was adequate and successful, since their responses, given in a five-point format ranging from Always to Never (5-Always, 1-Never), corresponded to positive results (≥ 3) in 83 to 100% of students. 83-100% of students in the teacher support dimension, 88-96% of students considered the good quality of the problem given and 100% of the students evaluated positively the dimension of student interaction and collaboration. Analyzing the responses to the questionnaire the average of the total score obtained by each student was 89 ± 16, which is in accordance with the interval defined previously by others corresponding to a minimum score of 23 and a maximum score of 115 (Senocak, 2009).

The analysis of the responses of students of self-perception of contributes to learning showed that students considered as corresponding to the higher contribute: the PowerPoint presentation and the hands-out containing the problematic situation (47% of the students), experimental work (41%) and computer simulation (12%).

The themes included in this teaching-learning strategy are relevant since United Nations defined the theme Water for Life for the decade 2005-2015 and the year 2015 as the International Year of Soils. Therefore, it is important to use this strategy in secondary education in order to increase the awareness about the scarcity of potable water, due to the expected rising of sea level, caused by climatic changes and the consequent increase in salinity of soils as well as contamination of underground potable water (Sousa, 2014; Sousa, 2015). Since this constitutes a global problem, it is useful to discuss the causes and consequences, as well as ways of reducing the negative impacts, such as the technological development of reverse osmosis processes for desalinization of sea water (UNESCO, 2014).

4. Conclusions

Since the aims of concept mapping and those of PBL are strategies that naturally complement each other in promoting meaningful learning (Addae et al., 2012), in our strategy we used PBL combined with two mapping phases. We also included some experimental activities, such as laboratory activities and computational simulations that were integrated in the context of implementation of PBL in a K–12 environment. Our results showed that we were able to encourage students' creativity and inquiry that are considered by several authors as essential for biology learning (DiCarlo, 2006), as well as critical thinking and questioning, as referred by students in their satisfactory feedback about the process, at the end of the corresponding unit. As observed by the satisfactory performance in the exam and lab report we also promoted the meaningful learning with long-term retention, and the ability to interpret laboratory results and its communication using drawings, graphically and in writing.

In conclusion, the application of the designed problem in high school promoted a multi-disciplinary discussion and constitutes a good example of STSE education and Socio-Scientific Issues discussion about the causes and consequences of environmental changes and ways of reducing their negative impacts (Ashraf, 2013; UNESCO, 2014). Therefore we presented a successful implementation case of integrated PBL in a public Portuguese school that may constitute an example to facilitate the implementation of active inquiry strategies by other teachers, as well as the basis for future research.

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