Journal of Advanced Veterinary Research

https://advetresearch.com

Cytotoxicity Screening of Anionic Dye Removal by Bio-Natural Adsorbent: Egg Shell and Peanut Shell

Parichat Srisamai[1,2], Prakaipet Pankaew[1], Poonnawis Sudtikoonaseth[2], Niwat Kangwanrangsan[1], Siriluck Iamtham[3,4,5], Wannee Jiraungkoorskul[1,*]

[1]*Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.*
[2]*Department of Dermatopathology, Institute of Dermatology, Ministry of Public Health, Bangkok 10400, Thailand.*
[3]*Institute for Advanced Studies, Center for Advanced Studies in Tropical Natural Resources, Kasetsart University, Bangkok 10900, Thailand.*
[4]*Department of Science, Faculty of Liberal Arts and Science, Program of Bioproduct Sciences, Kasetsart University, Nakhon Pathom 73140, Thailand.*
[5]*Laboratory of Genetics, Kasetsart University, Nakhon Pathom 73140, Thailand.*

ARTICLE INFO

Original Research

Received:
13 January 2021

Accepted:
19 March 2021

Keywords:

Agriculture, Dye, Environment, Quality of life, Wasting

ABSTRACT

Synthetic dye is produced by chemical compound that can be harmful to living microorganisms and human. Textile dye industry is inadequate dye effluent to the environment that can lead to water pollution. Therefore, the releasing dye effluent should be minimized. Eggshell and peanut shell adsorbent are recycled from solid agro-waste and household food waste. They are wildly used in adsorption process to dye effluent before releasing into natural water bodies. However, the toxic reduction of dye after dye removal is unknown. The aim of this study was to investigate the toxicity of eosin dye and its removal by adsorbent. Moreover, the cytotoxicity of dye is evaluated by brine shrimp lethality bioassay. The results showed that the dye after removal by eggshell had the lower mortality rate when compared to those of eosin dye and penut shell adsorption. The histopathological lesions such as abnormal appearance of enterocyte, blebing cell and coagulation necrosis were found. Therefore, these bio-natural adsorbents might be the alternative substances for the adsorption process in wastewater treatment and they might decrease the toxicity of dye pollution.

J. Adv. Vet. Res. (2021), 11 (2), 82-87

Introduction

A dye is a complex compound or colored substance, which is applied in the textile materials represent color. Dyeing wastewater causes the water pollution and effect on living organisms and ecosystems. For the example, eosin and methylene blue dyes can protect the penetration of light through water, which lead to a reduction in the rate of photosynthesis (Imran *et al*., 2015) and can decrease the dissolved oxygen levels, thereby affecting the entire aquatic biota (Hassan and Carr, 2018). Normally, there are two types of dyes; natural and synthetic dyes. The synthetic dye is generally dissolved in an aqueous solution, and it may be required a mordant solution to improve the fastness of the dye on the fiber in the textile industry or staining in tissue in the medical laboratory (Booth *et al*., 2002). Some synthetic dye can be carcinogenic,which can pose serious hazards to aquatic life. In this study, we used eosin (EO) for adsorbate as the model of anionic group. This dye is used for the toxicity test due to its biological staining and non-complete degradation (Hassaan *et al*., 2016) or difficult treatment (Ravi Kumar, 2000). This dye causes toxic, carcinogenic effects on aquatic biota including plants, animals and humans. The eosin dye may cause severe skin and eye irritation and has effect on vital organs (Sharma *et al*., 2008). When dye is directly contacted with the eye, it can cause injury to the cornea by destroying the retinal ganglion cell, located near the inner surface of the retina (Pavlidis *et al*., 2003). It can reduce the pulmonary gas exchange capacity (Sun *et al*., 1987) and its metabolites are also highly toxic and carcinogenic in nature (Borzelleca *et al*., 1987). In addition, EO dye is the water-soluble dye and applied directly to the neutral substrate (Benkhaya *et al*., 2017). Therefore, it is important to remove dyes from wastewater before their final disposal.

Technology for color removal can be divided into three categories: firstly, biological treatment is the wastewater treatment through bio-process or by using microorganisms to eradicate dissolve matters in wastewater and consequently, the dirt value would decrease. Secondary, chemical treatment, by which the dissolved inorganic components can be removed by adding an acid or alkali, by changing the temperature, or by precipitation as a solid. The precipitate can be removed by sedimentation, flotation, or other solid removal processes. Lastly, physical treatment that include the use of racks, screens, sedimentation, flotation, and filtration (Chigozie and Joseph,

*Corresponding author: Wannee Jiraungkoorskul
E-mail address: wannee.jir@mahidol.ac.th

ISSN: 2090-6277/2090-6269/

2014). However, the biological, chemical and physical processes are complicated and expensive, Moreover, chemical treatment might be contaminated from chemical substance when treatment processing. Therefore, the removal of dye is important for the wastewater treatment before discharge. It is difficult to remove the dye from the effluent because dye is not easily degradable and generally not removed from wastewater by conventional wastewater systems. In this study, the adsorption process, a part of physical wastewater treatment, is used. This adsorption process is occurred when substance in solution forming a molecular (adsorbate) accumulates on the surface of a solid or a liquid (adsorbent) for removing adsorbate from aqueous solution. It is widely used in the removal of contaminant from wastewater as a wastewater purification technique for removing a wide range of compound from industrial wastewater (Allen *et al*., 2005). The most commonly used adsorbent is the activated carbon and is particularly suited to the removal of polar compounds. The activated carbon can be made from wood, charcoal or coconut. Each type is characterized by a specific surface, grain size and pore diameter, which is used in powder form, granular form or in impregnated form (Brinkmann *et al*., 2016). However, the activated carbon use is usually limited due to the expensive production and the difficulty of disposing the used carbon (Boumchita *et al*., 2017). Therefore, the finding of a cheap or low-cost adsorbent is a good for an alternative way and more efficient materials for the wastewater removal. In the past few years, the number of researches have been studying the application of natural materials as economical adsorbents such as peanut, wood, clay, potato peel, egg shell and etc. (Boumchita *et al*., 2017) and involved bio-based materials, particularly to an agro-industrial waste such as fruit peel that is easily accessible in large quantities. The use of fruit peel as an adsorbent can be beneficial from economic and ecological points of view, since this material can be obtained at lower price, minimizing accumulation of agro-waste, providing possibility of regeneration of the adsorbent and the ability to extract metals from adsorbent (Meseldzija *et al*., 2019). Therefore, there is the need to develop the cheap cost effective method for the removal of these contaminants before discharge into the environment, for examples: agro-waste, natural waste and household waste.

Artemia salina or brine shrimp is an invertebrate zooplankton and is found nearly worldwide in saline lakes and pools (Lavens and Sorgeloos, 1996),it has inhabit inland saltwaters, such as brine pools and other highly salty habitats. The brine shrimp Artemia is a micro-crustacean, well adapted to the harsh conditions that severely hypersaline environments impose on survival and reproduction (Gajardo and Beardmore, 2012). It is widely used to detect cell toxicity (Meyer *et al*., 1982). Brine shrimp lethality bioassay is a simple test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp (Harwig and Scott, 1971). Examples of brine shrimp test, it is a preliminary toxicity screening of plant extracts (Ghosh *et al*., 2015), fungal toxins (Harwig and Scott, 1971), and heavy metals (Saliba and Krzyz, 1976). Sarah *et al*. (2017) had treated plant extracts with brine shrimp to check the toxicity. Lacave *et al*. (2017) had used brine shrimp for acute toxicity test of waterborne exposure to silver nanoparticles. In addition, brine shrimp can be associated with toxicity of dyes such as adsorption of methylene blue dye by using eggshell adsorbent and then, they were checked for toxicity with brine shrimp assay (Pankaew *et al*., 2019). Therefore, the brine shrimp is studied to several toxicity such as dyes, chemical substances, medical substances, plant extracts etc.

The objectives of this study were to investigate the cytotoxicity of eosin dye (EO) and its removal by adsorbent such as peanut shell (PS) adsorbent as a model of agricultural waste, and eggshell (ES) adsorbent as a model of household waste, which compared with activated carbon (AC) adsorbent. Moreover, the cytotoxicity of each group was evaluated by using brine shrimp lethality bioassay.

Materials and methods

Adsorbent preparation

The preparation of PS and ES powder were modified from Arunlertaree *et al*. (2007) and Massie *et al*. (2015). First, the peanut or egg were washed thoroughly with tap water to minimize their chromaticity and remove any dirties. Next, peanut was boiled in 100ºC for 30 min, but egg was boiled for 15 min, then removed and desiccated the shell. These shells were dried by hot-air oven at 65ºC for 24 h. The dried PS and ES were grinded by blender machine to fine powder, sieved through 2 mm diameter and stored in a glass container for further experiment. Activated carbon charcoal (AC) was used as a positive adsorbent model in this study. AC was grinded by blender machine to fine powder, sieved through 2 mm diameter and stored in a glass container.

Adsorbate preparation

EO, $C_{20}H_6Br_4Na_2O_5$ (Sigma-Aldrich, USA) as a model for anionic dye. The stock dye solution was prepared by dissolving 0.1 g of dye powder in 1.0 L of distilled water to give a concentration of 100 mg/L and diluted when necessary. The 20 mg/L concentration of dye solution was used to toxicity test and wavelength of EO was 523 nm in this study.

Absorptive removal dyes solution

This absorptive removal dye solution is the solution after dye removal which it was used in brine shrimp lethality bioassay. Firstly, the adsorbent dosages of dried PS, ES and AC powders were used in 5 g/L, and the concentration of adsorbate EO was initial concentration 20 mg/L. Next, they were shaken at speed 140 rpm by Orbit Shaker (VRN-480 Gemmy, Taiwan) for 60 minutes for the contact time experiment in room temperature. After shaking, the absorptive removal of dyes were separated by Whatman® Grade 1 (Merck, Thailand). Each of supernatant was measured by pH meter (Oakton PH 700 Benchtop, USA). Finally, this absorptive removal dye solutions were collected and treated with brine shrimp toxicity.

Artemia salina lethality bioassay

A. salina or brine shrimp is used for bio-maker cell toxicity test (Meyer *et al*., 1982). This study was divided to eight groups: Control group (3.5% NaCl), Adsorbate group (EO), Adsorptive removal solution (AC + EO), Adsorptive removal solution (PS + EO), Adsorptive removal solution (ES + EO), Adsorbent in distilled water (AC + DW), Adsorbent in distilled water (PS + DW), Adsorbent in distilled water (ES + DW).

Each group was in the condition of 3.5% NaCl. Ten brine shrimp in approximately equal size were exposed with 25 ml of the above. After 24 h exposure, the number of dead was counted and the percentage of death was calculated using Equation 1 (Sarah *et al*., 2017). The test was done triplicate and the brine shrimp was preserved in Davidson's fixative for histopathological process (Suvarna *et al*., 2018).

Brine shrimp death (%) = No. of dead brine shrimp/No. of dead + No. of live X 100 (1)

Histopathological analysis

Briefly, the specimens were washed with running tap water in order to remove formalin. They were dehydrated gradually with 70%, 80%, 90%, and 100% alcohol, respectively. Cleared from alcohol with xylene, then infiltrated with liquid paraffin. The paraffin blocks were prepared by using tissue embedding assembly. The paraffin blocks were sectioned at 5 microns thickness using a rotary microtome. The cut fine sections were placed in water bath at 40°C. So that it floats over the surface of water and folds were removed and then paraffin section was placed on a glass slide. Before any staining occurs, the slides were deparaffinized and running through the reverse process from xylene to alcohols to water. For staining, hematoxylin and eosin stain were used. The stained-glass slides were examined for tissue abnormalities using the light microscope and photographed by a digital. The ten fields in each slide were observed. The histopathological alterations were evaluated semi-quantitatively by ranking the lesion severity. Ranking from – to +++ depending on the degree and extent of the alteration as follows: (-) no histopathology; (+) histopathology in <20% of fields; (++) histopathology in 20-60% of fields; (+++) histopathology in >60% of fields. This ranking was modified from Benli *et al*. (2008).

Results

Artemia salina lethality bioassay

The percentage of death in Group 1 to Group 8 were 10%, 60%, 50%, 100%, 23%, 73%, 97% and 63%, respectively.

Histopathological analysis

Brine shrimp in Group 1 or control group showed the normal appearance of gastrointestinal system (Figs. 1A-1B). Brifly, enterocytes or intestinal absorptive cells were columnar shape with one nucleus and cilia on apex, they were arranged in one layer. Brine shrimp in Group 2 or EO was found with the disruption of membrane, swelling, blebing cells and abnormal nucleus: pyknosis, karyorrhexis and karyolysis patterns (Figs. 2A-2B). The absorptive removal solutions, Group 3 to Group 5, (AC+EO, PS+EO, and ES+EO, respectively showed the histopathological alteration such as hyperplasia cells, abnormal enterocytes, swelling cells, blebing cells and abnormal nucleus (Figs. 3A-3D). The adsorbent groups, Group 6 to Group 8, (AC+DW, PS+DW, and ES+DW, respectively), revealed the disruption of membrane, a few blebing cells and abnormal nucleus (Figs. 4A-5D). The severity of histopathological analysis in each group was given in Table 1.

Table 1. The severity of histopathological analysis in each group

Lesions	Gr.1	Gr.2	Gr.3	Gr.4	Gr.5	Gr.6	Gr.7	Gr.8
Blebing cell	-	+++	+	++	++	+	++	++
Hyperplasia	-	+++	+	++	-	+	+	-
Necrosis	-	+++	++	+++	+	+	+++	-

Gr.1: Control group (3.5% NaCl); Gr.2: Adsorbate group (EO); Gr.3: Adsorptive removal solution (AC + EO); Gr.4: Adsorptive removal solution (PS + EO); Gr.5: Adsorptive removal solution (ES + EO); Gr.6: Adsorbent in distilled water (AC + DW); Gr.7: Adsorbent in distilled water (PS + DW); Gr.8: Adsorbent in distilled water (ES + DW).

Note: Ranking from – to +++ depending on the degree and extent of the alteration as follows: (-) no histopathology; (+) histopathology in <20% of fields; (++) histopathology in 20-60% of fields; (+++) histopathology in >60% of fields.

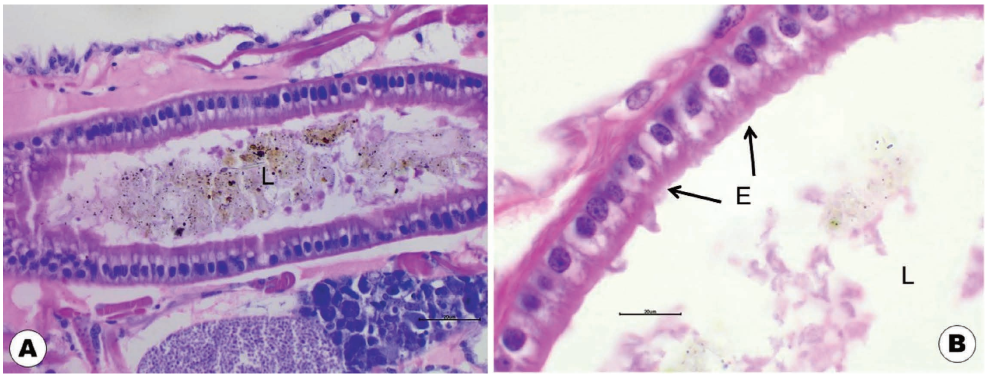

Fig. 1. Light micrograph of Group 1 or control group. (A) The midgut of brine shrimp was shown as normal gastric caeca or enterocytes (E) that composed in lumen (L), Obj. at 40x. (B) Higher magnification was shown enterocyte with cilia, Obj. at 100x.

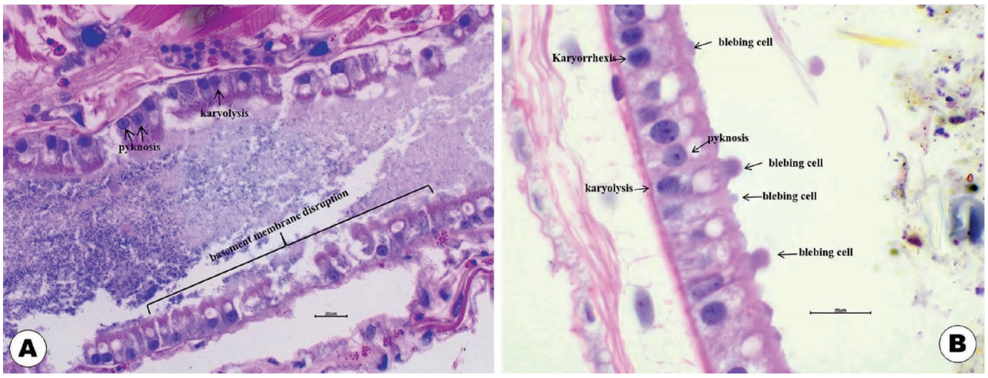

Fig. 2. Light micrograph of Group 2 or EO. (A) The midgut of brine shrimp was shown the basement membrane disruption, pyknosis and karyolysis. (B) It was found the lesions such as blebing cells, pyknosis, karyorrhexis and karyolysis, Obj. at 100x.

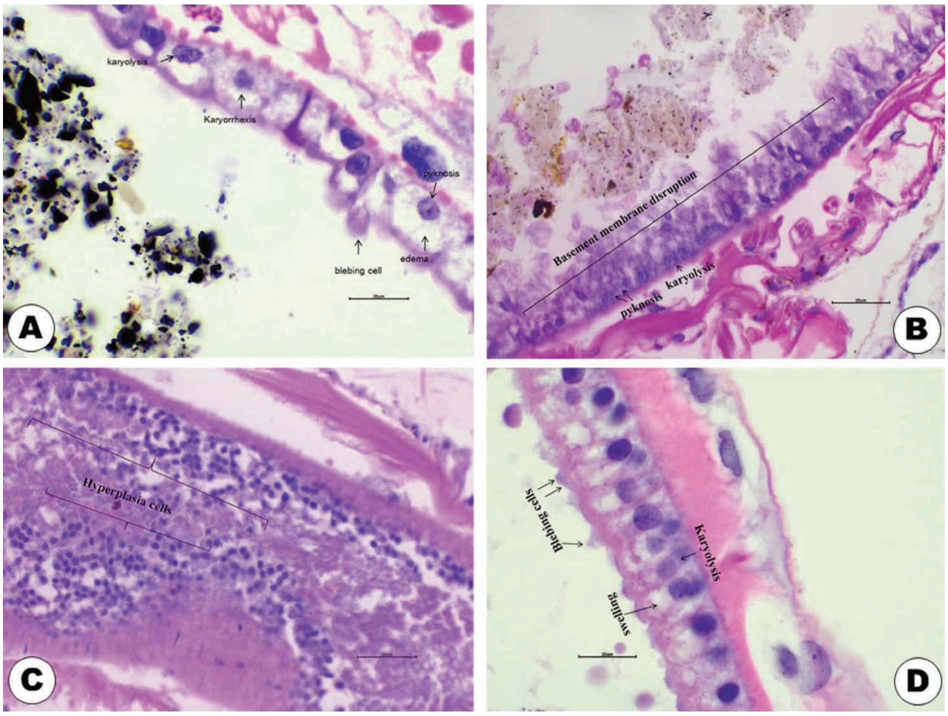

Fig. 3. Light micrograph of the absorptive removal solutions. (A) Group 3 or AC+ EO, it was shown belbing cell, pyknotic and karyolytic nuclei as well as accumulation of AC powder in lumen. (B-C) Group 4 or PS+EO, they were shown the basement membrane disruption, pyknotic and karyolytic nuclei. (C) Hyperplastic cells were found in some area. (D): Group 5 or ES+EO, it was shown swelling, belbing cell, and karyolytic nucleus, Obj. at 100x..

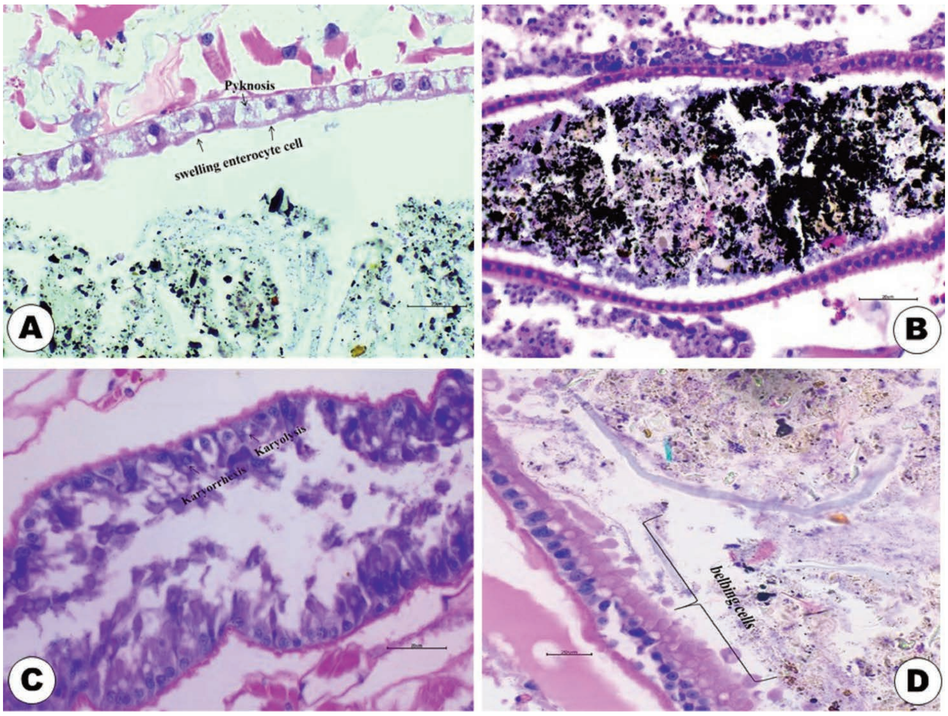

Fig. 4. Light micrograph of the adsorbent groups (A-B) Group 6 or AC+DW, it was shown the accumulation of carbon powder in lumen and also found swelling and pyknotic cells. (C) Group 7 or PS+ DW, it was found the irregular shape of enterocytes and coagulation necrosis in some area. (D) Group 8 or ES+DW, it was shown more blebing cells, Obj. at 100x.

Discussion

To the best of the authors knowledge, no report about the toxicity of dye and the absorptive removal solutions was published, so the present study was aimed to evaluate the cytotoxicity via brine shrimp lethality test. Even the commercial activated carbon is a popular adsorbent, but it is expensive and burning process add more dust into environment (Tucki *et al*., 2019). Therefore, this study points at the bio-natural adsorbent like the eggshell and peanut shell, which will be the alternative adsorbents, low cost and eco-friendly to the environment (Ikladious *et al*., 2017.) It has been reported that the ES and PS had the efficiency of dye adsorption from aqueous solution (Grag *et al*., 2019). The obtained results were shown that after adsorption of EO by adsorbent had decreased the toxicity in brine shrimp. The ES had very low mortality percentage at 23 while in AC had 50 percentage of death when compared with the EO dye, which had mortality percentage at 60. Therefore, the eggshell might be decreased the toxicity from eosin twice. Amarasinghe and Wanniarachchi (2019) mentioned that the eggshell is an eco-friendly environment and green number one in the industrial adsorption process. In addition, it has been reported that eggshell is a natural source for calcium and has been evaluated for safety in several animal and human studies primarily in Europe and Asia (Ruff *et al*., 2012). ES was tested with the acute oral toxicity in rats, and it said non-toxic. While activated carbon has effect on the respiratory system due to the burning process, which make more dust into environment, in addition, wet activated carbon depletes oxygen from the air (Tucki *et al*., 2019). In the present study, the accumulation of carbon powder was found in the lumen of brine shrimp as shown in Figs. 3 and 4, so that, the powder or dust cannot dissolve into aqueous solution. Although the peanut carries more benefits such as healthy food and rich protein but peanut shell had showed higher mortality of brine shrimp when compared with those of the eggshell, activated carbon or only eosin dye because it might be the ferment of peanut shell that made more acid situation during the removal dye process, also, the EO itself is an acid dye. Boumchita *et al*. (2017) reported the application of peanut shell as a low-cost adsorbent for the removal of anionic dye, at pH= 4, during adsorption process for 1 hour. The eosin dye is widely used in staining to differentiate bacterial species or for counterstain in histology (Bassey *et al*., 2012). The mucosal lining of the entire midgut (target organ) was composed of a single epithelium layer of cuboidal to columnar cells or enterocytes (Gunasekara *et al*., 2011). The present study has shown the histopathological lesions of enterocytes from adsorbate EO dye, adsorptive removal solution and adsorbent solution. The results revealed basement membrane disruption, swelling cell, blebing cell and coagulation necrosis including the various nucleus pattern such as pynkosis, karyorrhexis and karyolysis. The percentage of death of brine shrimp and the severity of histopathological analysis in eggshell group were low when compared with the other groups.

Conclusion

It can be concluded that eggshell as a bio-natural adsorbent might be an alternative choice for the adsorption process and it can reduce the toxicity of dye pollution.

Acknowledgement

This research was supported by Department of Medicinal Service Fund (Academic Support), Ministry of Public Health Thailand, in Academic Year of 2018. We would like to thank the members of the Fish Research Unit, Department of Pathobiology, Faculty of Science, Mahidol University, Thailand, for their technical support in the laboratory.

Conflict of interest

The authors declare that they have no conflict of interest.

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