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Histopathological Effects of Pulp and Paper Mill Effluent on the Digestive Glands of River Snails, *Filopaludinamartensi (Gastropoda, Viviparidae)* in the Mae Klong River, Western Thailand

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Abstract

The aim of this study was to determine the effects of pulp and paper mill effluent on the water quality and digestive glands of river snails (*F. martensi*) in the Mae Klong River. The water quality showed significantly (p<0.01) higher values for water temperature, electric conductivity, and total dissolved solids, as well as total suspended solids, biochemical oxygen demand and ammonia-nitrogen in effluent at downstream sites when compared to upstream sites. Histopathological analysis of the digestive glands of *F. martensi* identified effects ranging from slight to strong depending on distance from the source of pulp and paper mill effluent. Histopathological alterations consisted of enlarged tubule lumens, destruction and flattening of digestive cells, and irregular-shaped

basophilic cells, as well as enlarged hemolymph spaces between tubules, a breakdown of tubule basement membranes, and an increased amount of enlarged vacuoles. Flattening of digestive cells and dilated nuclei were also detected. The most violent symptoms were observed in the digestive glands of *F. martensi* collected from Site 3, located at the discharge point for pulp and paper mill wastewater.

Keywords: Pulp and paper mill effluent, water quality, *F. martensi*, histopathology, digestive gland

INTRODUCTION

Pulp and paper mill manufacturing is a leading environmental issue primarily due to the large amount of fuel, water and chemical resources consumed in the production processes. These processes cause the emission of environmental pollutants and negatively affect the environment through global warming, acidification, and eutrophication, as well as the creation of smog, toxicity and industrial waste [1]. The wastewater discharged from pulp and paper mills is of particular concern because it contains numerous pollutants from the various steps used in pulp and paper production. Wastewater also directly affects any receiving water [2]. Normally, pulp and paper mill effluent (PME) is subjected to secondary treatment before release into any aquatic environment [3]. However, more than 250 chemicals can be found in effluent from pulp and paper mills, creating a high risk for inadequate treatment [4]. The introduction of improperly treated pulp and paper mill effluent into a river results in increased algal nutrients (phosphorus and nitrogen). These nutrients have been shown to cause an enrichment effect, leading to enhanced productivity in the receiving water [5] as evidenced by the high concentration of chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), heavy metals and chlorophenol [6].

According to the aquatic biota used in ecotoxicological studies, invertebrates have been commonly employed because they are important in trophic chains and more sensitive to chemical pollutants [7]. Among invertebrates, snails are widely accepted as being an effective bioindicator of environmental stress. They are known to be potential bioaccumulation indicators of heavy metals [8, 9], responsible for insecticides [10] and phenol [11]. In snails, the digestive glands are the organs that play the most important role in the metabolism of endogenous and xenobiotic compounds [11, 12]. Therefore, altered histopathology in the digestive glands of snails is an important bioindicator of environmental stress [13].

River snails (*Filopaludina martensi* or Hoi Kom in Thailand) are classified into the Viviparidae family due to their soft tissue and operculum. They are an important source of protein and iron recognised as a fresh material in Thai cuisine. *F. martensi* has been found in various habitats including ponds, canals and river as well as other wetlands. The snails are commonly found in every part of Thailand [14]. Previous studies explored the use of *F. martensi* as a bioindicator focusing only on heavy metal toxicity [15-17]. As such, little information exists concerning the effects of other forms of pollution such as wastewater from pulp and paper mills. Therefore, study of the histopathological changes in *F. martensi* might be important as indicators of the effects of the effluent originating from pulp and paper mills [13]. Accordingly, the purpose of this study was to investigate the effects of pulp and paper mill effluent on the digestive glands of river snails, *Filopaludina martensi* by means of histopathological analysis.

MATERIALS AND METHODS

Water and River Snail Samples

Water samples and river snails (*F. martensi*) were collected from 6 study sites along the Mae Klong River in the Kanchanaburi and Ratchaburi Provinces of Western Thailand. Sites S1, S2, S3 and S4 were located in Kanchanaburi Province, while Sites S5 and S6 were located in Ratchaburi Province (Figure 1). Sites S1 and S2 were located upstream from a pulp and paper mill, while Site S3 was at an effluent introduction point. The remaining 3 sites were located downstream from a pulp and paper mill. Water temperature (WT), velocity, pH, electric conductivity (EC), total dissolved solids (TDS), alkalinity (AKL) and dissolved oxygen (DO) were measured in the field, while total suspended solids (TSS), biochemical oxygen demand (BOD_5) and nutrients were measured in a laboratory. River snails were collected from five stations along the Mae Klong River because Site 1 was absent of snails. Although 1 site was located upstream from a pulp and paper mill with effluent, four other stations were located downstream. The river snails had shells 2.5-3.0 cm in height and 2.0-2.5 cm in width. They were kept fresh in a cool box at a temperature less than 4 °C before dissection in the laboratory. The dissected digestive glands from river snails were fixed in Bouin's solution for at least 24 h before histopathology. After fixation, each digestive gland was embedded in paraffin and cut with a microtome. Subsequently, the sectioned specimens were strained with hematoxylin-eosin to study histopathological alteration.

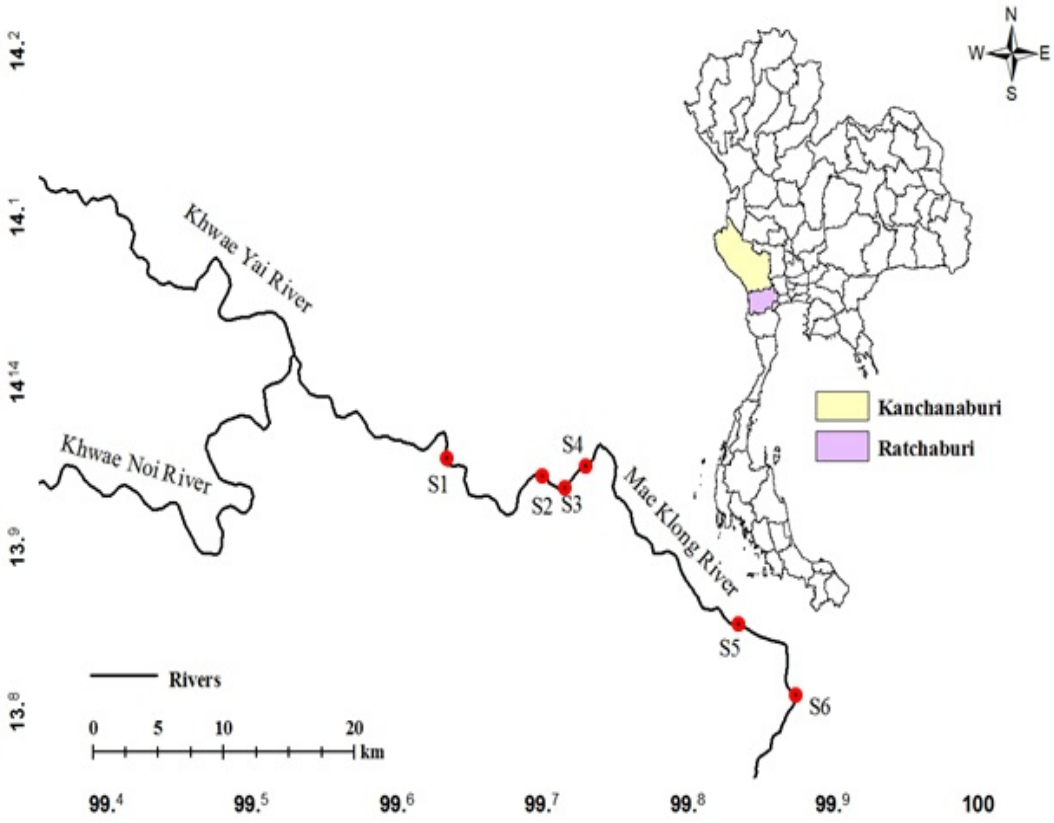


Figure 1: Six study sites along the Mae Klong River in the Kanchanaburi and Ratchaburi Provinces of Western Thailand.

DATA ANALYSIS

The results for physicochemical properties were presented as mean ± standard deviation (SD). Due to the lack of normal distribution for data, a Kruskal-Wallis test was used to check for significant differences between sampling sites. A biological assessment was completed to identify histopathological alterations in the digestive glands of the river snails.

RESULTS

Physicochemical Properties

The physicochemical properties for the Mae Klong River at each study site are shown in Table 1. All parameters showed significant differences between sampling sites, except for pH and soluble reactive phosphorus (SRP). Site S3 revealed significantly higher values for temperature, conductivity, TDS, TSS,

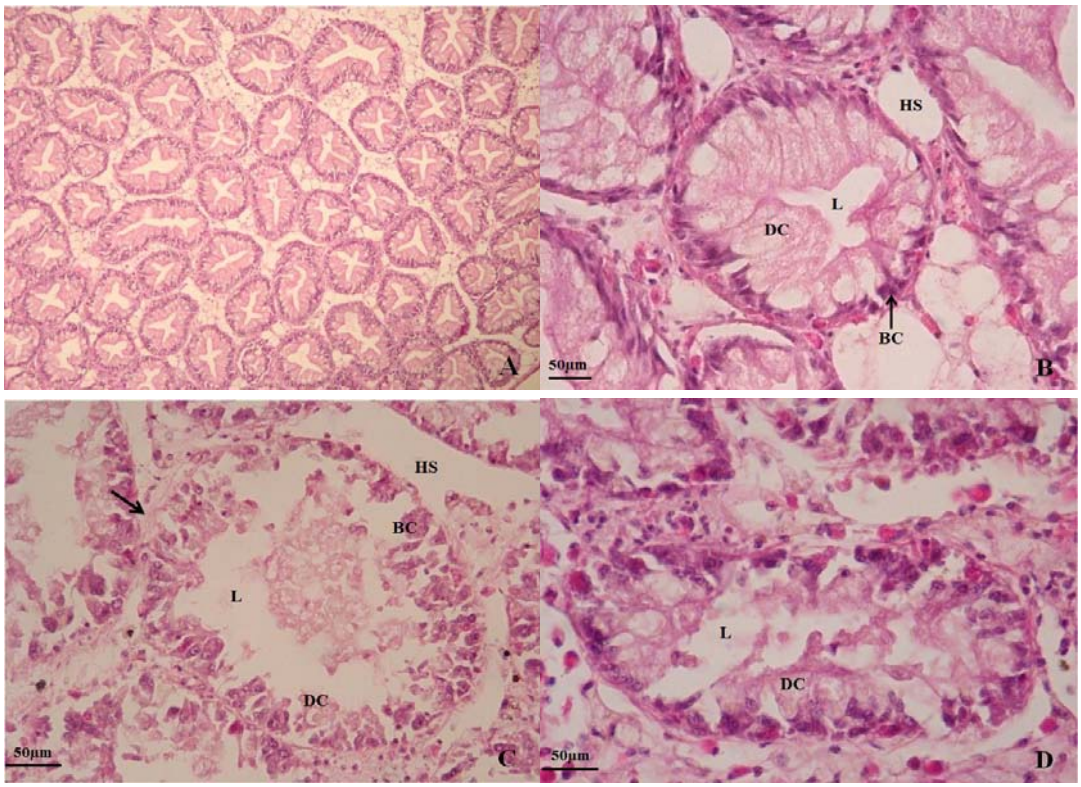
alkalinity, BOD_5 and NH_3-N compared to the other sites, although DO was the lowest (Table 1).

Table 1: Mean values (±SD) for the physicochemical properties at each sampling site during the entire sampling period. **P* <0.01; ***P* <0.001.

	S1	S2	S3	S4	S5	S6
WT (°C)*	29.43±1.21	29.08±1.83	32.0±0.00	29.09±1.37	30.58±2.04	30.73±2.44
Velocity (m/s)**	0.13±0.04	0.22±0.1	0.12±0.11	0.26±0.2	0.37±0.1	0.00
EC (µS/cm)**	174.68±28.5	195.73±1.45	1949.67±38.6	277.22±40.5	275.78±46.46	299.44±39.78
TDS (mg/l)**	94.04±2.81	101.66±6.09	998.33±45.81	141.22±18.19	140.67±21.28	152.89±17.97
TSS (mg/l)**	13.78±9.42	11.11±7.37	77.0±34.18	10.44±6.69	14.33±9.53	40.33±38.41
AKL(mg/l as $CaCO_3$)**	95±12.22	97.89±11.14	364.33±49.39	110.22±13.24	121.44±38.48	112±8.94
pH	6.96±0.27	7.1±0.18	7.05±0.25	6.97±0.1	7.19±0.39	6.98±0.26
DO (mg/l)**	6.1±0.43	6.82±0.76	4.79±0.82	6.6±0.84	7.58±0.32	8.07±0.43
BOD_5 (mg/l)**	0.77±0.33	1.19±0.75	4.19±0.74	0.69±0.3	0.9±0.52	1.34±0.49
SRP (mg/l)	0.25±0.24	0.23±0.14	0.26±0.09	0.17±0.1	0.22±0.15	0.17±0.08
NH_3-N (mg/l)**	0.17±0.11	0.13±0.08	0.90±0.36	0.07±0.04	0.18±0.09	0.18±0.09
NO_3^--N (mg/l)**	0.62±0.22	0.32±0.17	0.68±0.46	0.48±0.24	0.76±0.38	1.53±0.63

Histopathological effects of pulp and paper mill effluent

A total of 42 river snails were collected from 5 sampling sites along Mae Klong River. The histopathological alteration was presented higher at downstream sites compare to upstream site of the PME. The 100% of histopathological alteration of digestive glands were observed in the river snails collected from Site S3 S4, S5 and S6. Meanwhile, the unpolluted site (S2) was represented with 33.3% of histopathological alteration. The digestive glands of *F. martensi* from unpolluted sites were included with numerous digestive tubules having various shapes and sizes (Fig. 2A). The digestive tubules consisted of digestive cells, basophilic cells, tubule lumens and the connective tissue between the tubules (Fig. 2B). Histopathological alteration was observed in the digestive glands of snails collected from downstream sites where effluent was present. The most obvious and violent effects were observed in snails collected from Sites S3, S4 and S5, as displayed by enlarged tubule lumens, destruction of digestive cells, and irregular-shaped basophilic cells, as well as enlarged hemolymph spaces between tubules and a breakdown of tubules basement membranes (Fig. 2C-F). Meanwhile, snails collected from Site S6 showed an increased number of enlarged vacuoles, flattened digestive cells and dilated nuclei.



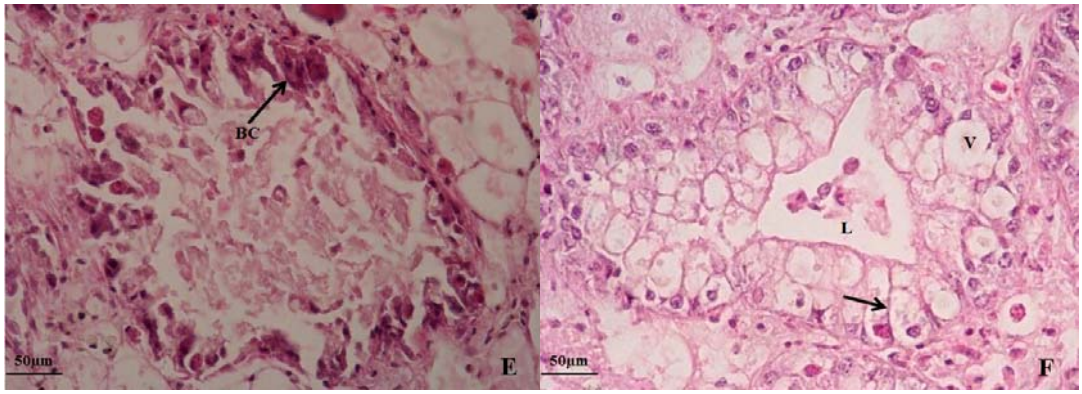


Figure 2: Light photomicrographs of the digestive glands of *F. martensi* showing A: digestive glands of *F. martensi* collected from unpolluted sites with digestive tubules; B: digestive tubules with digestive cells (DC), basophilic cells (BC) and tubule lumens (L). C: digestive tubules of river snails collected at effluent sites displaying enlarged tubule lumens, dilatation of intracellular spaces, loss of connective tissue, and increased hemolymph space between tubules as well as irregular digestive cells and destruction of tubule basement membranes (arrow); D-F: digestive tubule of *F. martensi* collected from downstream site of pulp and paper mill effluent destruction of digestive cells, flattened epithelials in digestive cells, dilated digestive cells (arrow) and increased various vacuoles.

DISCUSSION

The significantly higher values for water temperature, electric conductivity, TDS, TSS, Alkalinity, BOD, and ammonia nitrogen in addition to the lowest value for dissolved oxygen in Site 3 indicated the effects of pulp and paper mill effluent on the water quality in the Mae Klong River. This agrees with Usha *et al.*, [18], who studied PME and found higher values for electric conductivity, TDS, TSS and BOD. These results might be indicative of contamination in non-biodegradable organic material, absorbable organic halogens (AOX), phenolic compounds [19] and heavy metals [6] in the receiving water. Therefore, negative effects were caused directly to aquatic organisms, especially aquatic macroinvertebrates [20, 21].

Histopathological alterations in the digestive glands of gastropods have frequently been used as a bioindicator of environmental pollution [11, 13, 22-25]. In this study, histopathological alterations in the digestive gland tissues were observed in river snails collected from sites downstream of a pulp and paper mill known to possess effluent. The histopathological alterations included enlarged tubule

lumens, dilatation of intracellular spaces and irregular-shaped digestive cells, which were similar to previously reported findings in studies on ramshorn snails (*Marisa cornuarietis*) exposed to copper, lithium [13] and phenol on *Amphimelania holandric* Fér [11]. Meanwhile, the dilated nuclei were the same as the golden apple snail (*Pomacea canaliculata*) when exposed to sediment contaminated with heavy metals from the Beung Boraphet Reservoir in Thailand [26]. Further, increased vacuoles in digestive cells have been documented in *Lymnaeae stagnalis* exposed to Thaiodan® [10] and *M. cornuarietis* exposed to $PtCl_2$ [23]. The dilatations of digestive cells were similar to reported alterations in the digestive glands of *P. canaliculata* exposed to contaminated sediment from tributaries of the Mae Klong River [9].

The digestive glands of freshwater molluscs have been known to be the organ for detoxification, accumulation of toxicants, intracellular digestion, and metabolism of inorganic as well as organic compounds [25, 27, 28]. The histopathological alterations observed in the digestive glands of *F. martensi* collected from the Mae Klong River indicated the effects of pulp and paper mill effluent on aquatic macroinvertebrates, which might be potentially useful as a bioindicator of environment stresses.

CONCLUSION

The results of this study revealed that pulp and paper mill effluent has significant effects on the water quality of the Mae Klong River. Histopathological alterations in the digestive glands of *F. martensi* were apparent in snails collected from downstream sites containing effluent. This suggested that changes in the digestive glands of *F. martensi* are useful for studying the environmental stresses caused by pulp and paper mill effluent.

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