Çankaya University Journal of Science and Engineering
Volume 9 (2012), No. 1, 69–73

Histopathology of the Tissue of a Tubificid Worm (*Limnodrilus hoffmeisteri*) Exposed to Cadmium

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Özet. Kadmiyum herhangi bir organizma için gerekli bir element olmasa da oligocheateler -özellikle de Tubificid solucanları- diğer tatlı su örnekleri gibi kadmiyumu büyük yoğunlukta biriktirir. Bununla birlikte *Limnodrilus* cinsine ait olan bazı türler de indikatör organizmalar olarak kullanabilir. Bu çalışmada; farklı zamanlarda ve konsantrasyonlarda kadmiyuma maruz kalan tubificidler (*Limnodrilus hoffmeisteri*) deneysel olarak kontrol grubu ile karşılaştırılmış ve histopatolojik değişiklikleri incelenmiştir. Canlı örnekleri Porsuk Nehri'nin 3 farklı örnekleme alanından toplanmıştır. Örnekler iki farklı konsantrasyonda 0.25 ve 0.5 mg/L olarak 6, 12 ,24 ,48 ve 72 saatlik kısa sürelerde kirleticiye maruz bırakılmıştır. Örnekler parafin bloklara gömülmüş ve mikrotomda 5 mikron olarak kesilmiştir. Tüm kesitler Hematoksilen & Eosin ile boyanmıştır. Deney sonunda (6, 12, 24, 48 saatlik grublar) kontrol grubu ile karşılaştırıldığında değişiklik göstermemiştir. Ancak 72 saat süreyle kirleticiye maruz kalan gruplarda farklı sonuçlar gözlenmiştir.

Anahtar Kelimeler. Ağır metal, kadmiyum, histopatoloji, oligochaeta.

Abstract. Although cadmium is not an essential element for any organism, oligocheates - especially Tubificid worms - accumulate large concentrations of cadmium like other freshwater specimens. In addition, some species such as the ones that belong to *Limnodrilus* genera use indicator organisms. The aim of this study is to examine histopathological alterations induced experimentally by cadmium in tubificid worms (*Limnodrilus hoffmeisteri*) exposed for different times, and at different concentrations, and these were compared to controls. Live specimens were collected at 3 sampling sites from the Porsuk River. Samples were exposed to the contaminant for short periods of 6, 12, 24, 48 and 72 h at two different concentrations of 0.25 and 0.5 mg/L. Samples were embedded in paraffin blocks and were cut at 5 μm on a microtome. All sections were stained using Hematoxylin & Eosin. Our results showed no alterations (6, 12, 24, 48 h) compared with controls. However, after exposure for 72 h. results showed different alterations.

Keywords. Heavy metal, cadmium, histopathology, oligochaeta.

Received August 12, 2011; accepted May 15, 2012.

ISSN 1309 - 6788

1. Introduction

Cadmium, a non-essential metal that is toxic even when present in very low concentrations, is a relatively rare soft metal that occurs in the natural environment. It is known that Cd compounds can enter the bodies of aquatic animals via the gills, the general body surface and the alimentary canal, following ingestion of contaminated food particles [1]. The impact of pollutants on living systems can be evidenced at different levels of biological organization, from molecules and cells to communities and ecosystems [2]. Several benthic invertebrates, such as chironomids and tubificid worms, are closely associated with surficial sediments, burrowing in the upper layer and feeding on particulate matter. Trace metals can be taken up by these benthic organisms and included in the food-chain [3,4,5]. Although several studies on metal accumulation by benthic organisms from river sediments have been conducted, evidence of histopathological damage to digestive system cell of Tubificid worm due to cadmium exposure is still scarce. The objective of this study was to determine Cd-induced histopathological alterations in tubificid worms, sampled from 11 water courses in four river basins with different physical and chemical characteristics.

2. Materials and Methods

Living Tubificid worm samples (*Limnodrilus hoffmeisteri*- Tubificidae, Oligochaeta) were collected at 3 sampling sites of the Porsuk River basin in Eskişehir (Türkiye) in the summer and autumn seasons of 2007. Tubificid worms retained on the 500 μm sieve were collected and rinsed with deionised water. Samples were exposed to the contaminant for short periods of 6, 12, 24, 48 and 72 h at two different concentrations of 0.25 and 0.5 mg/L. Samples were not fed during the experiment period and were fixed with a buffered 10% formaldehyde solution pH 7.2 and kept until their examination in the laboratory. Samples were dissected and the processing of samples for histopathological study was as follows. Samples were bathed three times in alcohol at 96% (2 h each) three times in butylic alcohol for 24 h each and, finally, they were embedded in paraffin three times for 3 h each. Samples were embedded in paraffin blocks and were cut at 5 μm on a microtome [2]. All sections were stained using Hematoxylin & Eosin. Our results showed no alterations (6, 12, 24, 48 h) compared with controls. However, after exposure for 72 h, results showed different alterations.

3. Results and Discussion

In the alimentary canal cells of control *L. hoffmeisteri* samples, both secretion cells and digestive cells are recognized with the presence of lumen secretion. Digestive cells showed basal nucleus and secretion cells showed a normal voluminous nucleus cells (Figure 1(a) and (b)). The alterations of the alimentary canal cells of *L. hoffmeisteri* changes as a result exposure to Cd.

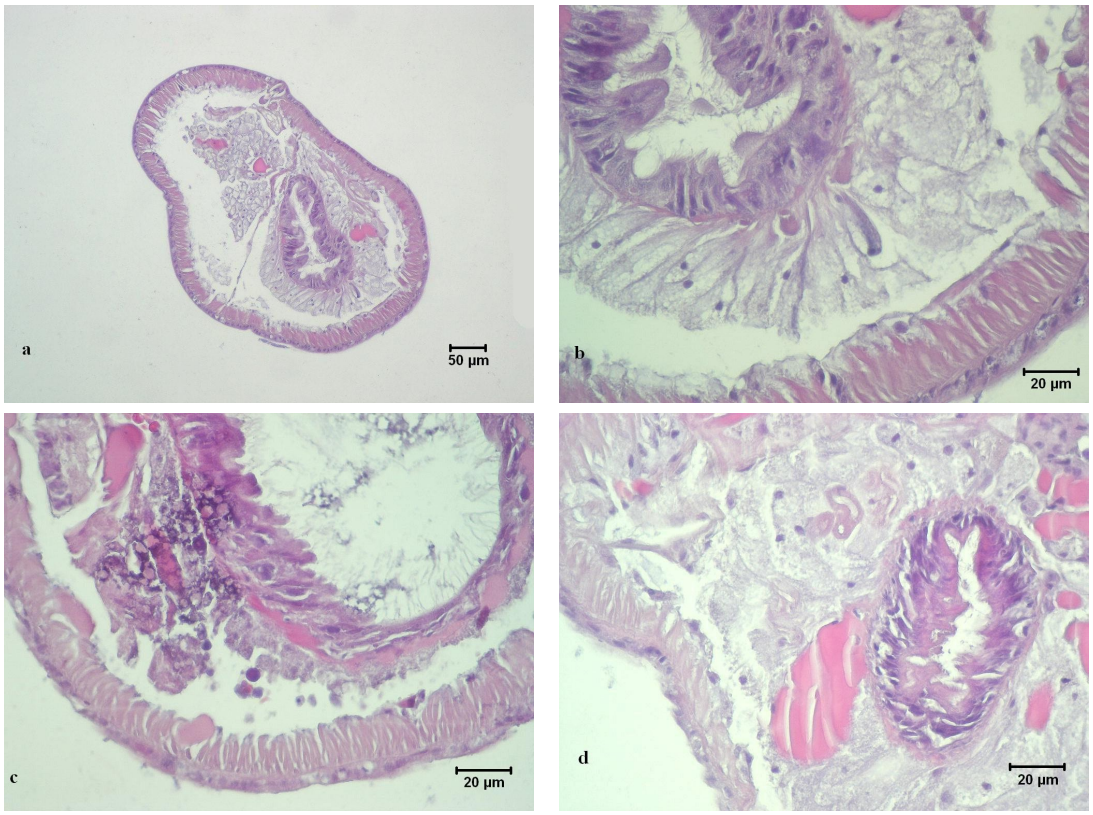

FIGURE 1. (a), (b) Control samples paraffin section of alimentary canal of *L. hoffmeisteri*; (c) paraffin section of alimentary canal exposed to 0.25 mg Cd L^{-1} for 72 h; (d) paraffin section of alimentary canal exposed to 0.50 mg Cd L^{-1} for 72 h.

The present study showed that change in alimentary canal cells depended upon the external Cd concentration.

The structure of epithelial cells, especially in the alimentary canal system, showed different changes with different concentrations of exposure to Cd. Samples were exposed for 6,12, 24, 48 and 72 h at both toxicant concentrations (0.25 and 0.50 mg/L). Samples exposed for 6,12, 24 and 48 h at 0.25 mg/L and 0.50 mg/L of Cd

showed no alterations compared with controls. However, for 72 h, at 0.25 mg/L of Cd they showed few alterations compared with controls while for 72 h at 0.50 mg/L of Cd they demonstrated significant alterations. The histological state of all the specimens' cells from the alimentary canal system exposed for 72 h at 0.25 mg/L vacuolization was observed, with a reduction in the number of secretion cells and epithelial cells.

Figure 1(a) and (b) show examples of histological section samples of the control group and Figure 1(c) illustrates alterations in alimentary canal system cells (0.25 mg/L, 72 h). As seen from Figure 1(c), the epithelium layer of the digestive system of the sample exposed to 0.25 mg/L cadmium for 72 h was thinner than that of the control. It is known that the secretion cells have a characteristically pyramidal shape and contain apical spherules of protein material and vacuoles with phosphorous-rich granules in the median region of the cell [6]. In the present study, it was observed that their characteristic shape was demorfed after being exposed to 0.25 mg/L cadmium for 72 h.

For all specimens exposed for 72 h at 0.50 mg/L alterations which can be called autolysis were observed in all cells of the alimentary canal system (Figure 1(d)). At 0.50 mg/L, secretion cells become indistinguishable from digestive cells, which show no structural integrity. Our results indicated that exposure to 0.50 mg/L Cd causes the breakdown of digestive cells and this is evidenced by an increasing occurrence of intense vacuoles. It can be concluded that cell deformation and damage of the digestive system depend on concentration. A relation was observed between the exposure concentration and the deformation of secretion cell and occurrence of intense vacuoles. In addition, secretion cells also undergo gradual changes which involve an increase in cell size.

Our observations are in agreement with previous studies [7,2]. In addition, several studies have ranked the Tubificid worms as the animals which can be used most successfully as a biological monitor of contaminants in the aquatic habitat. Previous investigations [8,9] on the freshwater or earthworm also demonstrated its capability for heavy metal storage. The freshwater tubificid *Limnodrilus hoffmeisteri* is capable of bioaccumulating cadmium several times more than that of the surrounding water and sediment. Fast uptake processes lead to fast equilibration of the internal body concentrations. Light microscopic examination of freshwater tubificid worms (*Limnodrilus hoffmeisteri*) collected from Porsuk River, which is a relatively polluted river, showed demonstrable changes in the histological structure

of the alimentary canal cells. Finally, a certain level of cadmium must be present in the tissues to give rise to 0.50 mg Cd L^{-1} for 72 h morphological changes.

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