

# Biomarker Response in the Polychaete *Nereis succinea* as Early Signal for Heavy Metals Pollution in the Red Sea, Egypt

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## Abstract

The polychaete annelids (family nereidae) are abundant in intertidal zone. These animals have potential to accumulate and tolerate some toxic metals in their tissues. In the present study, the four heavy metals; Mn, Pb, Cd and Cu in the whole body of the polychaete worm *Nereis succinea* sampled from two areas were measured. The concentration of heavy metals determined for individuals inhabited both studied sites has confirmed the potential of the selected worm to accumulate heavy metals from ambient habitat. On the other hand, histopathological investigations have clarified that the granular deposition of heavy metals within tissues of *N. succinea* was related to the degree of heavy metals loads from site to another.

**Keywords:** Red sea; *Nereis succinea*; Heavy metals; Biomarker; Histopathology

## Introduction

Aquatic pollution is one of the issues that have gained a worldwide attention. Environmental pollutants impact not only threats the biotopes, but also deteriorates the human health [1]. Regarding aquatic pollution, both marine and fresh water eco systems are subjected to different types and sources of pollution. Among the most hazardous pollutants are heavy metals. The risk of heavy metals is being in the fact that these substances cannot be degraded or removed via biological processes and magnify in the food web [2-6].

The determination of metal concentration within tissues is the appropriate method to evaluate the bioavailability and the effective metal concentration in water. Therefore, it's easy to measure the heavy metal concentrations regardless of its amount in the natural environment [7,8]. The levels of biomagnification of heavy metals in food web containing fish for example as consumer were higher several folds than those in the surrounding habitat [9-11].

The polychaete *Nereis succinea* is benthic and thereby will expose to high concentrations of heavy metals from sediments and interstitial water. Such benthic organisms are intermediate links in the marine foodweb. The polychaetes are capable of accumulation of heavy metals from their environment via different routes including ingestion of sediment and subsequent accumulation over gut epithelia as well as from pore water and overlying water via cutaneous uptake i.e. diffusion over the body surface [12]. The process of metal uptake is governed by many factors such as bio-ecological and physiological activities of organisms and species [13,14]. A good biomonitor organism is that can provide data and information about its ecosystem [7,10,11,15-17].

The selected organism selected for biomonitoring must accumulate contaminants several folds of their persistence in habitat. In addition, these accumulators should detoxify or convert the toxic substances into non-harmful stored compounds. Previous works have indicated

the use of polychaetes for biomonitoring, since some polychaetes such as *Nereis succinea* possess the criteria of sentinel organisms that enable them to be the appropriate biomonitors [8,10,11,18].

The present study aims to compare the concentration of heavy metals; Mn, Cd, Cu and Pb at soft tissues of *Nereis succinea* inhabited marine biological station MBS and fish port station FBS. Histopathological studies will be conducted for individuals collected from two sites to evaluate the effect of accumulated heavy metals on gut tissues of studied individuals.

## Materials and Methods

### Study area and sampling protocol

Two sites were chosen for the present study. The first one was the Marine Biological Station (MBS), and the second site was the Fishing Port at Sakala (FPS). Both sites (Figure 1) are located on the Red Sea coast at Hurghada city (Red Sea governorate).

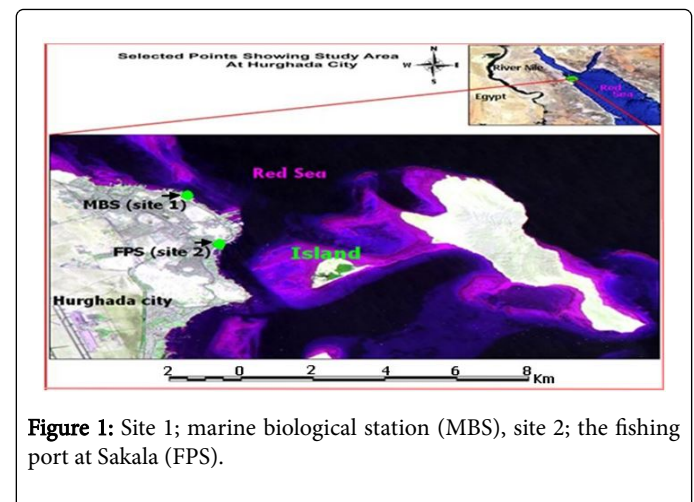


Figure 1: Site 1; marine biological station (MBS), site 2; the fishing port at Sakala (FPS).

## Sampling

Worms were picked from sediments by hand. Worms were transferred into a container filled with sea water. To keep the body shape straight and somewhat to remain more uniform, worms were anaesthetized by adding drops of ethanol till specimens are relaxed, these should be added gradually (a few drops at a time). Then worms were transferred into a container and fixed with 10% normal formalin. After 24 hours, animals were ready to be transferred to 70% ethyl alcohol for identification and histopathological studies [19].

For metal analysis, worms were kept in freezer after transported to lab in ice bags. Heavy metals in tissues were determined according to [7,8,10,11].

For light microscopy, ethanol preserved worms were dehydrated, cleared, then embedded in paraffin and processed routinely for light microscopy. Tissues were sectioned at 5  $\mu$  and stained with haematoxylin and eosin (H&E) [20].

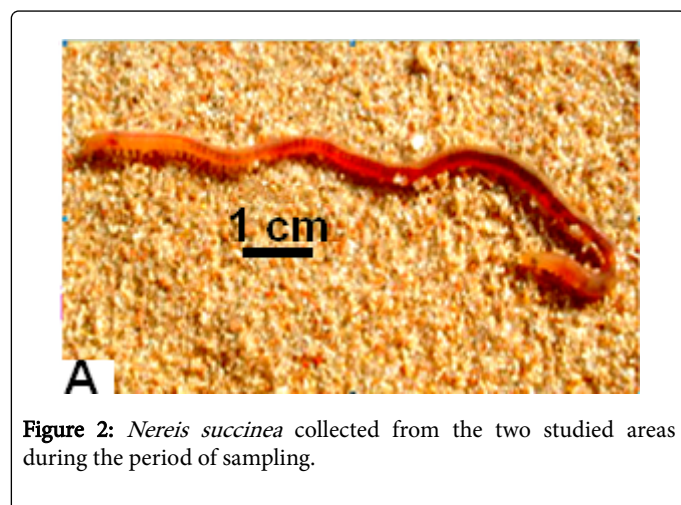
Sections were examined by light microscope and photographed. Head (with appendages) and parapodia were stained with acetocarmine stain prepared according to the method of [18].

## Identification of polychaete species

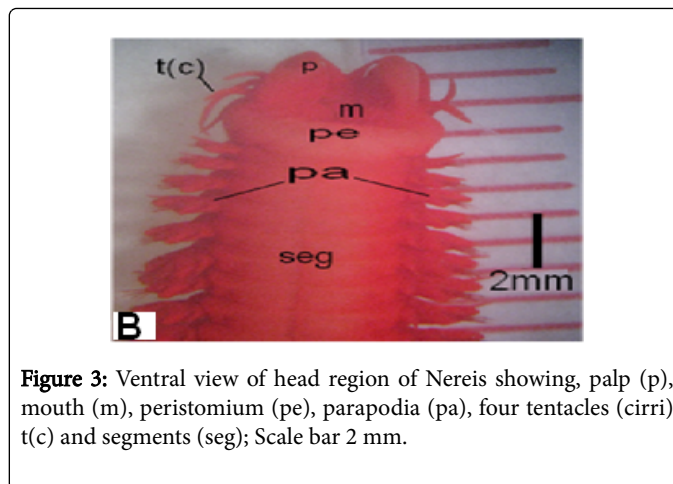
For identification, all measurements were carried out using a scaled ruler on a binocular microscope according to [21,22]. Adult worms were chosen, "Adult females" are defined as individuals possessing microscopically visible coelomic gametes. Measurements of body width are performed without parapodia according to [23].

The common morphometric characters as width or length of setiger (segments bearing parapodia) width or length of the prostomium and the anterior part of the body or size used in identification according to [24-26].

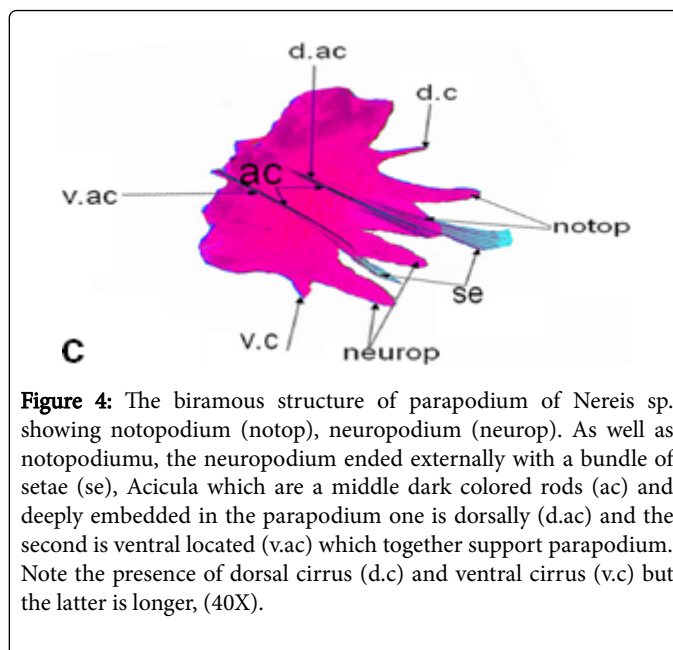
Because doubtful differences in morphological characters in the collected polychaetes were present, other groups of the same samples were sent to the "Educational Museum of the Egyptian Fauna in Zoology Department Faculty of Science Assiut University, for accurate identification for each site collected samples (Figures 2-4).



**Figure 2:** *Nereis succinea* collected from the two studied areas during the period of sampling.



**Figure 3:** Ventral view of head region of *Nereis* showing, palp (p), mouth (m), peristomium (pe), parapodia (pa), four tentacles (cirri) t(c) and segments (seg); Scale bar 2 mm.



**Figure 4:** The biramous structure of parapodium of *Nereis* sp. showing notopodium (notop), neuropodium (neurop). As well as notopodium, the neuropodium ended externally with a bundle of setae (se), Acicula which are a middle dark colored rods (ac) and deeply embedded in the parapodium one is dorsally (d.ac) and the second is ventral located (v.ac) which together support parapodium. Note the presence of dorsal cirrus (d.c) and ventral cirrus (v.c) but the latter is longer, (40X).

## Results and Discussion

Annelid worms were previously used as bioindicators for their inhabitant aquatic ecosystem. Bervoets et al. [27] used Tubificid worms as predictors for ecological changes in aquatic ecosystem. The present survey had revealed that two studied sites are different in their ecology. MBS is a protected area away from human activities and sources of pollutions. In contrast the 2<sup>nd</sup> site (FPS) is impacted by several activities of fishing. The annelid polychaetes belong to family nereidae and represented the same specie *Nereis succinea*. It has been noted that the second site is richer in nutrients in the term of eutrophication.

Consequently, animals of the second site are healthier than those of the first one. Surprisingly, the annelids from the second site are slightly shorter than the opposite population. To explain that, in previous work conducted by [19] on *Nereis diviricolor* from two different sites in pollution degree, they found that *Nereis diviricolor* was differed significantly from clean to polluted habitats.

Authors [19] attributed these morphological differentiations to the nature of the two areas. We must take in consideration that disturbed ecosystems tend to be characterized by small reselected species [26]. In the current work, the polychaete *Nereis succinea* could take up and accumulate higher heavy metals concentrations (Table 1 and Figures 4 and 6) from the 2<sup>nd</sup> site than the 1<sup>st</sup> one.

The mean values of manganese ( $0.87 \pm 0.56$ ), cadmium ( $0.85 \pm 0.33$ ) and copper ( $0.95 \pm 0.13$ ) were more than three folds in the 2<sup>nd</sup> site when compared with those of the 1<sup>st</sup> site; ( $0.18 \pm 0.02$ ), cadmium ( $0.26 \pm 0.23$ ) and copper ( $0.31 \pm 0.34$ ). In fact sediments of FPS are muddy and finer than those of MBS. Sediments can be considered as a sink for many pollutants, thus the accumulation of heavy metals there represent the past and present accumulations. Selck and Forbes [12] stated that More than 90% of the Cd body burden in the polychaete *Capitella* sp. was resulted from sediment-associated pool of Cd.

It is easy for aquatic organisms to take up heavy metals from their habitat via the active transport though their permeable bodies [15]. Metal ions have affinity for proteins and other cellular constituents. The active transport pump through carrier protein has an active role as route of entry of some metals. Free cadmium ion for example, has a similar radius to that of calcium and will be taken up through the calcium [28,29].

The observation of granular depositions in epithelial layer lining the gut of *Nereis* collected from FPS might be attributed to metal storage, this finding agrees with [8,10]. Many phyla could store such granules in their bodies [30].

This work is in agreement with [26,31] who revealed the presence of accumulated copper in tissue of *Nereis diversicolor* appeared as green-black deposited granules in the epidermis of the body wall and parapodia. Mild ulceration in the lining of gut has been observed in animals collected from the second site.

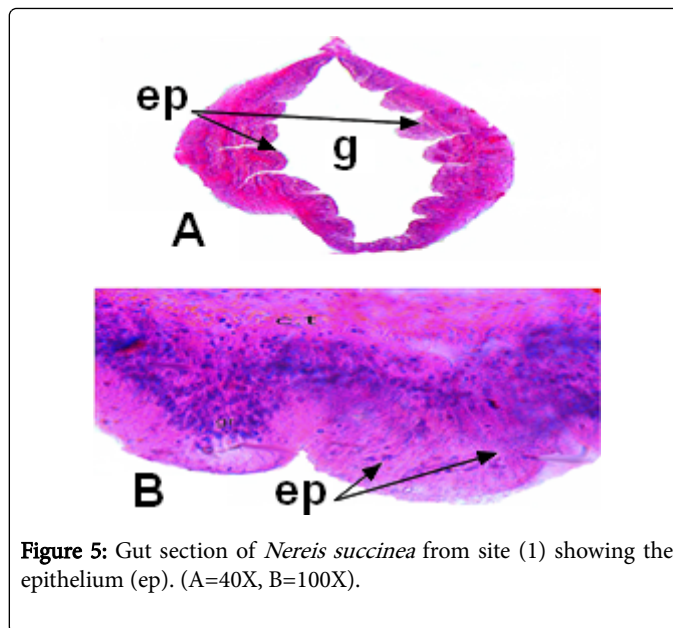
The deterioration of guts is a result of compartmentalization of granular metals in gut epithelium. Author observed the accumulation of zinc in barnacle *Elminius modestus* as detoxified pyrophosphate granules. In the fresh water *Hyridella depressa*, the microscopic examinations have revealed the presence of granular aggregations in different tissues.

The presence of granules at the gut epithelial cells (Figure 5) may be attributed to the intake sediments adsorbed heavy metals on their surfaces. At the time of digestion, the adsorbed metals are collected at the epithelial cells as granular structures [28,29,32,33]. In fact, the granular aggregations were previously thought to play roles in removal of toxic metals [15].

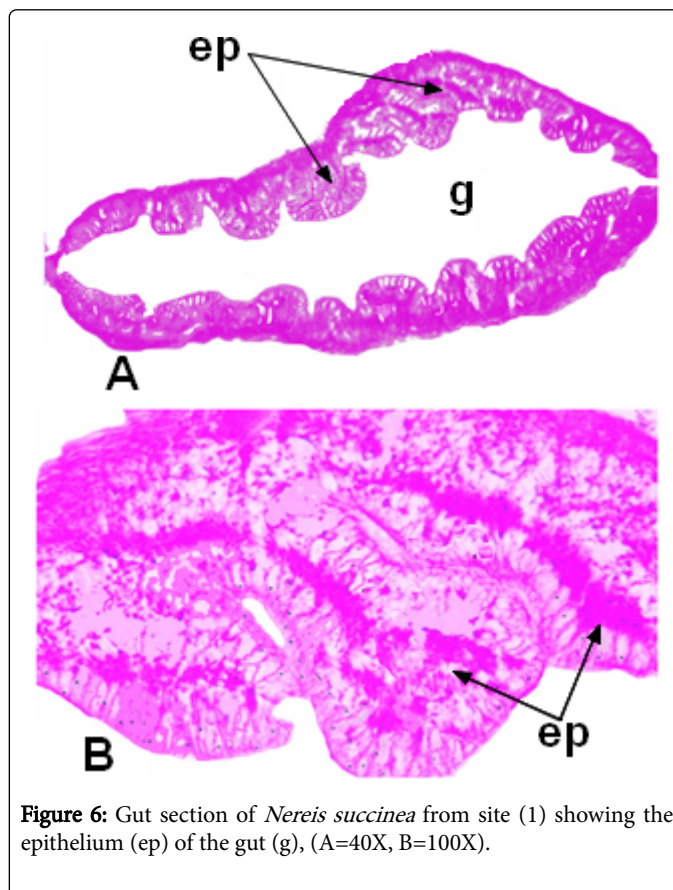
Future studies are needed to further investigate ecological biotic and abiotic factors that control bioaccumulation of heavy metals within soft tissues of polychaete worms.

Site	Mn	Pb	Cd	Cu
Site 1	$0.18 \pm 0.02$	$0.59 \pm 0.46$	$0.26 \pm 0.23$	$0.31 \pm 0.34$
Site 2	$0.87 \pm 0.56$	$0.78 \pm 0.67$	$0.85 \pm 0.33$	$0.95 \pm 0.13$

**Table 1:** Heavy metal concentrations ( $\mu\text{g/g}$ ) in tissue of *Nereis* at the two sites.



**Figure 5:** Gut section of *Nereis succinea* from site (1) showing the epithelium (ep). (A=40X, B=100X).



**Figure 6:** Gut section of *Nereis succinea* from site (1) showing the epithelium (ep) of the gut (g). (A=40X, B=100X).

## Conclusion

The light microscope examinations of *Nereis* gut has demonstrated a high accumulation capability of the worms collected from polluted site (2) compared with that of worms collected from relatively clean site (1).

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