# Immune Response in Mussels to Environmental Pollution

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RECENT studies have used the blue mussel, *Mytilus edulis*, as a sentinel organism for pollution (Greig & Sennefelder 1985; Micallef & Tyler 1989). The mussel and other marine mollusks, such as oysters and clams, have long been known to concentrate toxic heavy metals, such as mercury, lead and cadmium. They are also capable of bioaccumulating synthetic organic compounds, such as PCBs and pesticides. This propensity to concentrate toxins often poses a public health problem, but it also offers an opportunity for environmental monitoring. The United Nations Environmental Program has in fact launched a global effort to monitor these organisms for marine pollution (Bayne 1989; Beeby 1993; Borchart et al. 1988).

Not only can mussels act as reservoirs to measure the extent of chemical contamination and its variation in different coastal environments, it can also be used in the teaching lab to illustrate biological responses to pollution (Bayne 1989; Conrad 1988). Pollutants, such as heavy metals, have been found to alter molecular and cellular processes in mussels. While each organism is unique in both its capacity to deal with pollutants and its biological responses to them, certain cellular responses are often similar down the phylogenetic scale. A serious concern in recent years is the immune suppressive effect that pollutants are having on marine life. This phenomenon has been observed from invertebrate organisms, such as mussels and oysters, to vertebrates such as fish, seals and whales (De L. Swart et al. 1994; Faisal et al. 1991; Sami et al. 1992).

The blue mussel offers an easy way of introducing your class to both immune response and the effects of environmental pollution on marine organisms. The immune cells of mollusks are called hemocytes and can be easily extracted with hemolymph from the mussel's foot or heart with a syringe. These cells have been shown to have many characteristics of vertebrate granular leukocytes. They respond to the same messenger molecules as leukocytes and perform a similar phagocytic function (Stefano et al. 1989b).

We have shown in our laboratory that hemocytes of the blue mussel respond to heavy metals by rounding up and becoming inactive. This leads to a suppression in their ability to phagocytize bacteria and other foreign objects such as latex beads. One measure of immune suppression that your students can perform is to count the number of cells that have phagocytized bacteria or beads after being incubated with the foreign object and varying concentrations of a heavy metal as compared to a control. The bacteria offer a more realistic function of the immune cells but require a sterile technique. The latex beads (0.1$\mu$, Sigma) are a convenient substitute. Both stain a dark purple with methylene blue and contrast nicely with the hemocyte.

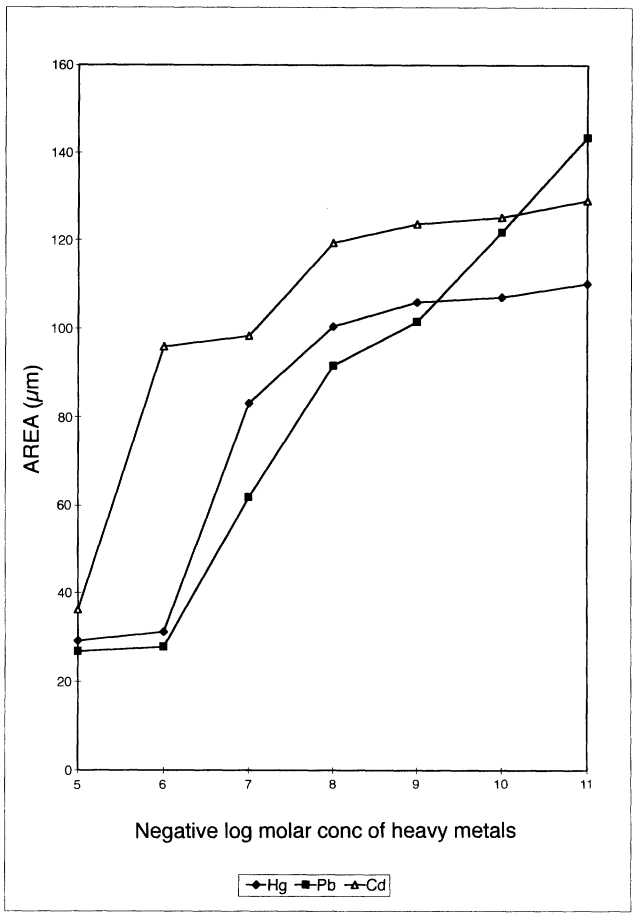


Figure 1. Effect of heavy metals on area.

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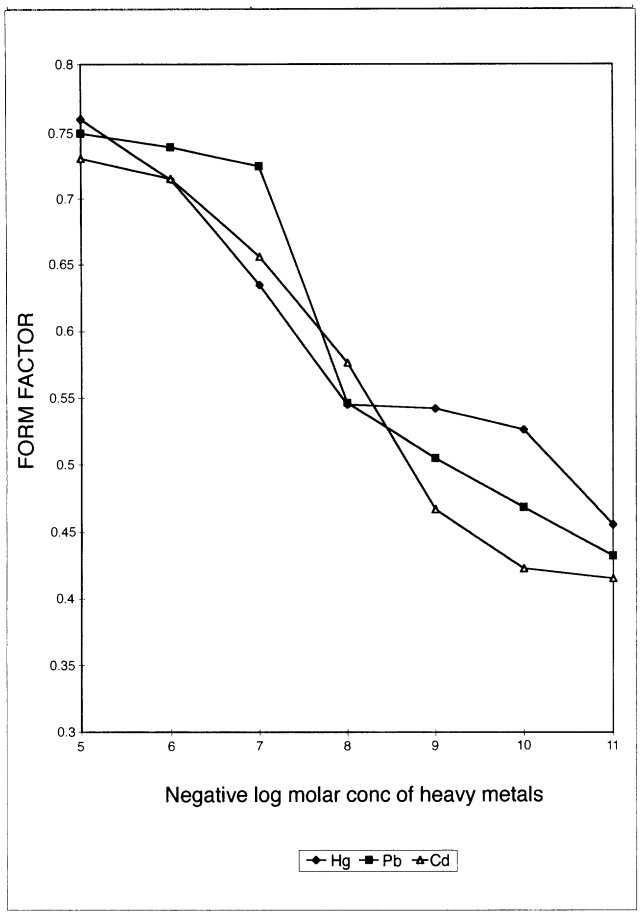


Figure 2. Effect of heavy metals on form factor.

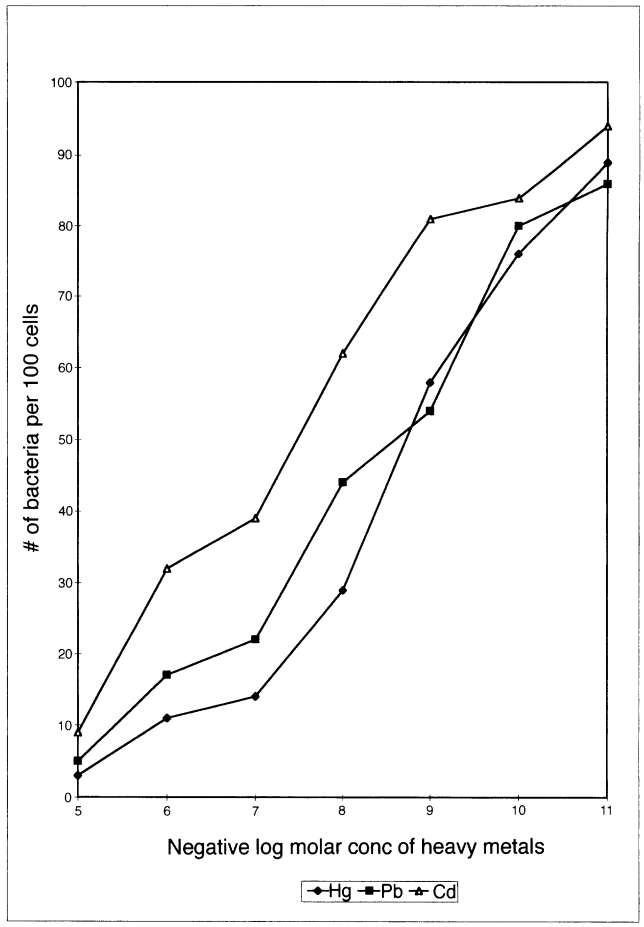


Figure 3. Effect of heavy metals on latex bead phagocytosis.

Another procedure that can be performed on mussel hemocytes, illustrating immune suppression, is the measurement of shape and area of the cells using computer-assisted microscopy. Recently, a user friendly, reasonably priced system has become available from the Image Analytics Corp. of Hauppauge, NY. This system, called Image 100, employs a microscope, video camera, and personal computer to make these measurements instantaneously. The investigator focuses a slide of the cells under the microscope that appears on the computer monitor. Using a mouse, an outline of each cell is made. The computer calibrates the area and the shape of the cell using a form factor of $(4 \times \pi \times \text{area/perimeter}^2)$ where a value of 1 is perfectly round. An immune stimulated cell becomes larger and more amoeboid while an immune suppressed cell tends to round up and become smaller. The data collected can be easily transported to a spread sheet where statistical tests can be performed.

The response of hemocyte area and shape to concentration of mercury, lead and cadmium is shown in Figures 1 and 2. Figures 3 and 4 show the phagocytosis of latex beads and bacteria, respectively. The best concentrations of metals to show an effect in contrast to controls are in the middle range of response. Too much of a concentration causes the cells to become compressed little balls. It is of more interest to show a suppressive effect at low concentrations than to merely show that metals are toxic to cells. This reflects the type of immune suppression that one observes in marine organisms at even low levels of pollution.

This procedure can also be used to demonstrate immune suppression of other substances, including polycyclic aromatic hydrocarbons. However, salts of metal are more easily obtained than many of the more exotic hydrocarbons of interest and are relatively harmless if used properly. There are many other experiments that one can do with mussels but most require a greater investment in time and equipment. For a good review, try B.L. Baynes' "Measuring the Biological Effects of Pollution: The Mussel Watch Approach" (1989). This laboratory should prove a good introduction to biological monitoring for your students.

## Procedures

*Mytilus* samples must be collected fresh and in good health. A mussel in good health will close quickly when touched (if open) or will be difficult to pry open (when closed).

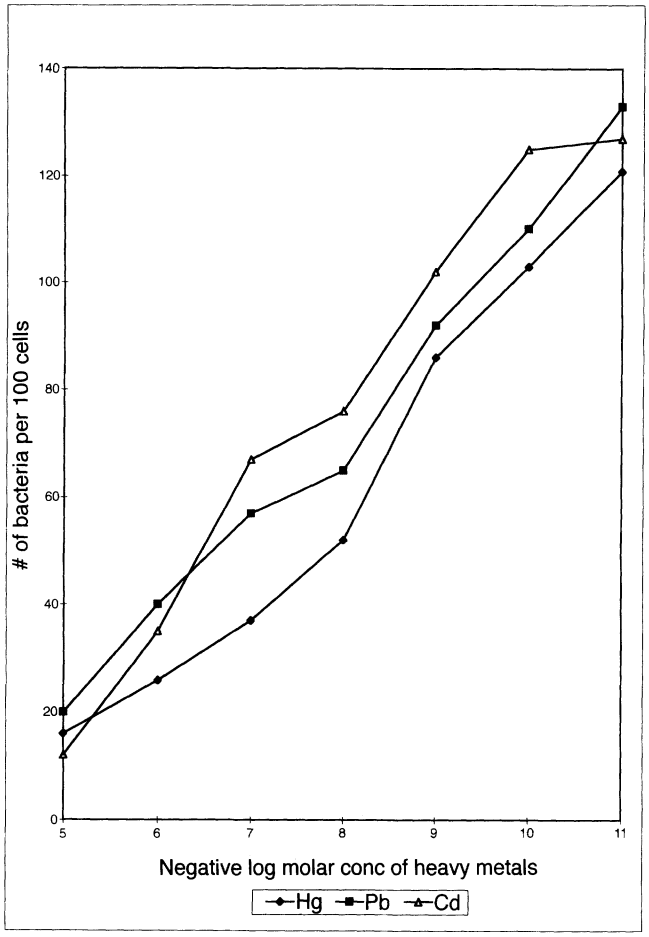


Figure 4. Effect of heavy metals on bacteria phagocytosis.

Before extracting the hemocytes, prepare slides on which to place them. A thin ring of histological mounting media (Permamount), smaller than the cover slip is applied to the face of the slide to both enclose the hemolymph and create a tight seal when the coverslip is in place.

To extract hemolymph from *Mytilus*, hold the mussel at the narrow end with the convex side of the shell placed downward. Pry open the wide, convex end with a scalpel, gently twisting the blade to keep the bivalve open. With the mussel open, extract 0.1 ml of hemolymph from the posterior adductor muscle, being careful not to pierce through this muscle as this will dramatically increase the number of bacteria in the hemolymph, impairing the analysis.

Place one or two drops of the hemolymph inside the ring and add the desired concentration of heavy metal. After a 15-minute incubation period, add the bacteria or latex beads. At this point, place the slides in a humid container. After another 15-minute incubation period, stain the slide for one minute with methylene blue. After rinsing off the stain, place a drop of mounting media within the ring and drop the coverslip in place. The slide can now be examined under the light microscope at 400× power.

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