NONPOINT SOURCE POLLUTION LESSON PLAN

The Seeds Tell the Story

Focus

Bioassays to measure toxicity of nonpoint source pollution

Grade Level

9-12

Focus Question

How can students measure the toxicity of nonpoint source pollution?

Learning Objectives

- Students will describe at least five sources of nonpoint source pollution runoff.

- Students will describe and discuss at least five actions that can be taken to reduce or eliminate contaminated runoff.

- Students will define and discuss the meaning of toxicity, dose, sensitivity, and route of exposure.

- Students will explain the concept of a "dose-response relationship," and discuss why this relationship may not be adequate to define "safe levels" of potentially toxic substances.

- Students will explain why bioassays may provide a more realistic picture of toxicity than chemical analyses alone.

Materials Needed

- Copies of "Radish Seed Bioassay Worksheet," one copy for each student or student group
- Ziplock plastic bags, at least 12 per student group, depending upon the number of control solutions required (see Learning Procedure, Step 3)
- Paper towels, one for each plastic bag
- Permanent markers for labelling sample jars and plastic bags
- Radish seeds, 10 per plastic bag
- Household bleach

- Forceps, one for each student group
- Glass containers for sample collection (volume determined in Learning Procedure, Step 1)
- Graduated cylinder (approximately 50 ml), one for each student group
- Protective gloves and safety glasses, one set for each student handling samples

Optional items needed only if students sample from brackish waters (see Learning Procedure, Step 3):

- Hydrometer, artificial seawater salts, beakers and graduated cylinder for preparing artificial seawater

Audio/Visual Materials Needed

None

Teaching Time

Two or three 45-minute class periods, plus time for students to observe bioassays over a 5-day period

Seating Arrangement

Groups of 3 – 4 students

Maximum Number of Students

30

Key Words

Nonpoint source pollution
Sediment
Bioassay
Toxicity
Dose-response

Background Information

Pollution is the presence of substances in the air, land, or water that can degrade human health and environmental quality. These substances may come from many sources, but some of the most serious pollution problems are the result of unwanted by-products (commonly called “wastes”) from human activities.

In the early 1970's, major steps were taken to reduce pollution from human activities, including passage of the Clear Air and Clean Water Acts and establishment of state and federal environmental protection agencies. Many of these efforts were targeted toward large, conspicuous sources of pollution such as factories and municipal sewage systems, and significant progress has been made in reducing pollution from these "point sources." Point source pollution is pollution that comes from a single, identifiable source such as a pipe or smokestack. But many polluting substances do not originate from a single source. Rainwater, for example, may become contaminated as it moves over and through the ground, picking up pollutants from many different sources. This kind of pollution is known as nonpoint source pollution, and now accounts for most of the water pollution in the United States.

Pollutants from nonpoint sources include:

- fertilizers and pesticides from farms and home landscapes;
- oil, grease, and toxic fluids from roads, parking areas, leaking underground storage tanks, and improper disposal of used motor vehicle lubricants;
- sediments from poorly managed construction sites, forest lands and stream banks;
- acid drainage from abandoned mines; and
- bacteria and nutrients from livestock, pet wastes, and faulty septic tanks.

In fact, nonpoint source pollution comes from such a wide variety of human activities that almost everyone contributes to the problem in some way, often without realizing it.

Because nonpoint source pollution is a serious and pervasive problem that costs millions of dollars in lost and damaged resources, degrades environmental quality, and threatens human health, it has been the focus of numerous state, local, and national efforts. The diversity of sources and substances that result in nonpoint source pollution often make it difficult to know exactly what actions are needed to reduce or eliminate the problem. In many cases, the first priorities are to recognize when pollution is taking place, identify the polluting substances, and determine the sources of pollutants. These tasks are part of the mission of NOAA's Center for Coastal Monitoring and Assessment (CCMA). Through the National

Status and Trends Program, CCMA conducts long-term monitoring of toxic chemicals and environmental conditions at more than 350 sites along the the U.S. coasts. Monitoring typically includes measurements of:

- the concentrations of common pollutants in sediments and the tissues of living organisms;
- the toxicity of sediments to living organisms; and
- the abundance and variety of species in bottom (benthic) communities.

Often these three types of information are combined to obtain an overall indicator of environmental quality. This method is known as the sediment quality triad approach.

This activity is designed to acquaint students with bioassays (tests that use biological organisms to study the action of chemicals or physical changes in the environment), and to provide hands-on experience with a simple bioassay that students can use to study nonpoint source pollution and many other aspects of environmental quality.

Learning Procedure

1.

To prepare for this activity, assemble all materials needed for bioassays, and determine the volume of water needed to saturate the paper towel to be placed in the zip-lock bag. This will vary depending upon the size and composition of the paper towel. The towel should be uniformly damp, but there should not be a pool of water in the bag. Students should collect sample volumes approximately four times the volume needed to saturate a single paper towel.

2.

Discuss the concept of nonpoint source pollution, or have students complete one version of the Nonpoint Source Pollution Self Test. Lead a short brainstorming session to identify some common sources of nonpoint source pollution in the students' community.

Briefly discuss the concept of a pollutant. Be sure students understand that this term can apply to almost anything that is "in the wrong place at the wrong time in the wrong quantity" and has the potential to harm the environment or human health. Ask students what makes something toxic or poisonous? One of the fundamental concepts of toxicology was stated by a 16th century Swiss physician and alchemist named Paracelsus (pronounced "para-SELL-suss"; his original name was Theophrastus Bombastus von Hohenheim) who basically said "The dose makes the poison." In toxicology, dose is the amount of exposure to a potentially toxic substance, and depends upon the concentration of the substance, the duration of the exposure, and the size of the organism being exposed to the substance. The key implication of Paracelsus' statement is that everything is poisonous if the dose is high enough. How an organism responds to a potentially toxic substance also depends upon the sensitivity of the organism to the substance (all substances are not equally toxic to all organisms) and the route of exposure (whether the substance is inhaled, absorbed through the skin, eaten, injected, etc.)

3.
Introduce the concept of bioassays as a way to measure toxicity. Tell students that they will be using radish seeds as a bioassay to investigate nonpoint source pollution in their community. Two responses will be investigated: seed germination and root growth. Have each student group identify three potential sources of nonpoint source pollution that they can sample for their investigation, and submit these for your approval. Emphasize that because these samples may contain substances that are harmful to human health, it is imperative that students wear protective gloves and safety glasses while handling their samples.

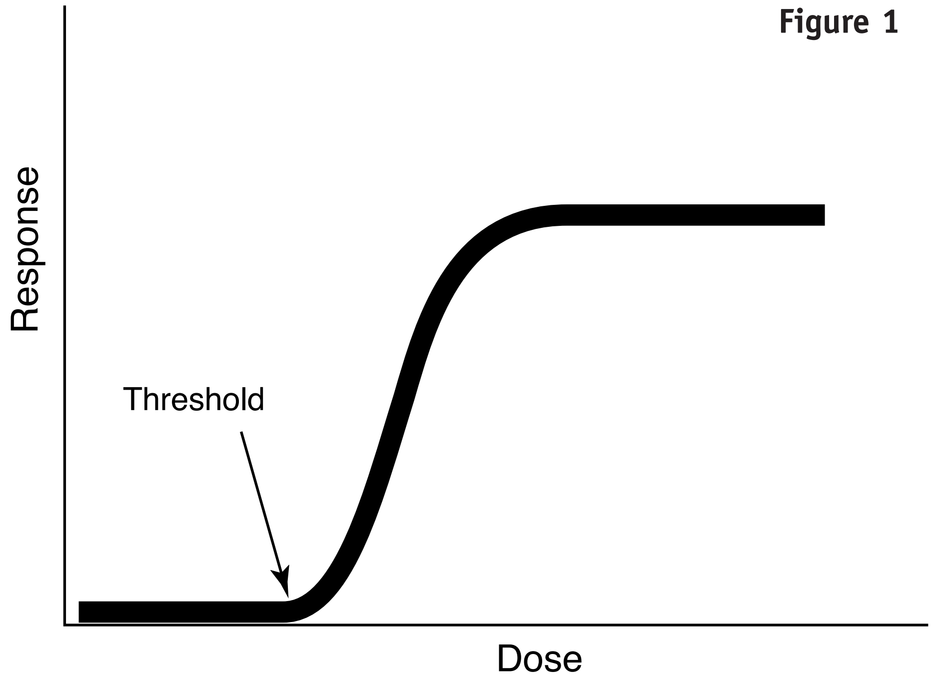
Students should also identify an appropriate control for each of their samples. If samples are from freshwater sources, distilled water would be an appropriate control. If the samples are from brackish water, it will be necessary to prepare control solutions that have approximately the same salinity as the sample. The easiest way to do this is to measure the specific gravity of the sample with a hydrometer (available from aquarium supply stores). Then prepare a solution of artificial seawater (also available from aquarium supply stores) and dilute the solution to the same specific gravity as the sample. If sample solutions have different specific gravities, a separate control solution should be prepared for each sample.

4.
Have each student group collect samples (volume determined in Step 1) from their approved sources and prepare appropriate control solutions. Remind students to label sample bottles with the sample location, collection date, and sample number. Remind students to keep a notebook in which they record details of their sample collection, laboratory procedures, and results. Provide each group with a copy of the "Radish Seed Bioassay Worksheet" and instruct students to complete the bioassays for their samples as directed on the worksheet. Be sure to tell students how much sample should be placed into each bag. Each group should prepare a written report of their results, including a discussion of the outcome.

5.
Have each group present an oral discussion of their investigation and results. Lead a discussion of students' results. Students should realize that different organisms are not equally sensitive to chemical agents. For example, the concentration of copper in water that would kill algae or a snail is harmless to most fish. When choosing a bioassay organism, investigators need to consider which compounds or organism responses are of most concern. Seed bioassays are very sensitive to herbicides and fairly sensitive to metals. They are less sensitive than fish or invertebrate assays to industrial chemicals like PCBs or solvents. A full evaluation of a sample's biological activity requires performing several different bioassays (see the accompanying lesson, "Get to the Point!" for an example). Bioassays for drug screening, for example, often include bacteria (to screen for potential antibacterial activity) and specific tissue cultures (to screen for anti-cancer activity).

It is important that students understand two points. The first is the meaning of the dose-response relationship, which is basically a description of how an organism responds to a substance as the exposure to the substance increases. This relationship is often used as the basis for establishing safe levels of potentially harmful substances. The idea is that if response is graphed as a function of exposure, there will be a threshold below which no response can be measured, followed by a range of exposures over which response increases to a point at which it reaches a maximum. Beyond this point, increasing

Figure 1



exposure does not produce any further increase in response (Figure 1). The problem is, this standard concept of the dose-response relationship doesn't represent how organisms actually respond to many chemicals. Even within the same species, individuals can vary widely in their response to various substances. People with allergies can have severe reactions to quantities of contaminants that would produce no response in other individuals. Fetuses, babies, and children are also much more sensitive to many contaminants than adults. Another problems is that some dose-response relationships appear to have no thresholds at all; in other words, any exposure produces a response. For example, a study of the effects of estradiol (a sex hormone) found that every dose tested altered the sexual development of turtles, even though the smallest dose was on the order of 0.0000000004 (four hundred trillionths) gram per egg (Sheehan, *et al.*, 1999). Other dose-response relationships are inverted, so that smaller doses produce greater effects than larger doses. For example, bisphenol A (used to manufacture polycarbonate plastics and epoxy resins found in drinking water containers, baby bottles, reusable food containers, coatings inside food cans and dental sealants) has been found to change the developmental rate of mouse embryos and alter the structure of breast tissue in a way that is associated with breast cancer, and these responses are more pronounced at low doses of the chemical than at higher doses (Takai, *et al.*, 2001; Markey, *et al.*, 2001).

The second point is that the toxicity of nonpoint source pollution is not just the toxicity that results from individual contaminants; it is the toxicity of a particular combination of contaminants. These combined effects are known as synergistic effects. This point is critical when trying to decide how polluted a particular environment may be, because chemical analyses typically list the concentrations of individual chemicals or groups of chemicals without any information on how these substances may interact. For example, a study of the effects of four pesticides on human breast cells showed that low concentrations of these chemicals (too low to cause a response by themselves) when mixed together caused a response that was similar to the effects of estrogen. In other words, harmless amounts of four chemicals added up to a dose that could cause harm (Payne, *et al.*, 2000). For more information about the problem of natural and man-made chemicals that mimic the action of human estrogen and may be linked to an increasing incidence of endocrine-related cancers and deteriorating reproductive health in humans and wildlife see Wolff, 1995 and Toppari *et al.*, 1996 (see Resources).

An obvious limitation of students' investigations is that no tests were performed to identify the substances that may have produced toxic effects on the radish seeds. This would be critical if one wanted to identify the sources of toxicity so they could be remediated, but it is often a complex task because nonpoint source pollution typically includes more than one type of contaminant. In fact, because of the issues of variable dose-response, synergistic effects, and multiple contaminants, bioassays may provide a more realistic picture of toxicity than chemical analyses alone.

The Bridge Connection

http://www.vims.edu/bridge/ – In the "Site Navigation" menu on the left side of the page, click on "Ocean Science Topics," then "Human Activities," then "Enviro-concerns," then "Pollution" for links to resources about marine pollution.

The Me Connection

Have students write a brief essay describing the most significant sources of nonpoint source pollution in their own communities, what specific personal activities may contribute to contamination from these sources, and what they might do to reduce this problem.

Extensions

1. Visit *http://oceanservice.noaa.gov/education/kits/pollution/supp_pollution_roadmap.html* for links to additional information and activities related to nonpoint source pollution

2. Visit *oceanservice.noaa.gov/education/classroom/09_coast_manag.html* for an activity in which students construct a model watershed to demonstrate and study nonpoint source pollution.

3. Have students investigate nonpoint source pollution issues in their own watershed. A good place to start is *http://cfpub.epa.gov/surf/locate/index.cfm*, which is a gateway to individual watersheds and various databases containing relevant water quality information.

4. Nosocomial infections (infections caused by microorganisms that are resistant to antibiotics and other antimicrobials) in hospitals or hospital-like settings are responsible for over 100,000 human deaths per year. One of the ways that microorganisms develop this type of resistance is through exposure to antimicrobials that are widely prescribed by physicians and veterinarians and that enter the environment through nonpoint and point sources. Visit *http://oceanexplorer.noaa.gov/explorations/04etta/background/antimicrobial/antimicrobial.html* for more discussion of this problem, and *http://oceanexplorer.noaa.gov/explorations/04etta/background/edu/media/bump.bugs.pdf* for a lesson plan on this subject.

5. Visit *http://www.epa.gov/owow/estuaries/monitor/* for a manual on volunteer water quality monitoring in estuaries.

Resources

http://www.coastalmanagement.noaa.gov/resource.html#education – NOAA Office of Ocean and Coastal Resource Management, Resources, Publications and Outreach Materials

http://www.epa.gov/win/ – U.S.E.P.A. Watershed Information Network

http://www.epa.gov/owow/nps/eduinfo.html – U.S. EPA Web site with education resources about polluted runoff

Payne, J., N. Rajapakse, M. Wilkins, and A. Kortenkamp. 2000. Prediction and Assessment of the Effects of Mixtures of Four Xenoestrogens. Environmental Health Perspectives 108:983–987.

Wolff, M. S. and P. G. Toniolo. 1995. Environmental Organochlorine Exposure as a Potential Etiologic Factor in Breast Cancer. Environmental Health Perspectives 103(Suppl 7):141-145.

Toppari, J., J. C.. Larsen, P. Christiansen, A. Giwercman, P. Grandjean, L. J.. Guillette Jr., B. Jégou, T. K. Jensen, P. Jouannet, N. Keiding, H. Leffers,J. A. McLachlan, O. Meyer, J. Müller, E. Rajpert-De Meyts, T. Scheike, R. Sharpe, J. Sumpter, and N. E. Skakkebæk. 1996. Male Reproductive Health and Environmental Xenoestrogens. Environmental Health Perspectives 104(Suppl 4):741-803.

Sheehan, D. M., E. Willingham, D. Gaylor,, J. M. Bergeron, and D. Crews. 1999. No Threshold Dose for Estradiol-Induced Sex Reversal of Turtle Embryos: How Little Is Too Much? Environmental Health Perspectives 107:155-159.

Takai Y, O. Tsutsumi, Y. Ikezuki, Y. Kame, Y. Osuga, T. Yano, and Y. Taketan. 2001. Preimplantation exposure to bisphenol A advances postnatal development. Reproductive Toxicology 15(1):71-4.

Markey, C. M., E.H. Luque, M. Muñoz de Toro, C. Sonnenschein and A. M. Soto. 2001. In Utero Exposure to Bisphenol A Alters the Development and Tissue Organization of the Mouse Mammary Gland. Biology of Reproduction 65:1215–1223.

Rathbun, J. 1996. A Simple Bioassay Using Lettuce Seeds. The Volunteer Monitor 8(1):70-72 (available online at *http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon08no1.pdf*)

National Science Education Standards

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard B: Physical Science

- Chemical reactions

Content Standard C: Life Science

- Interdependence of organisms

Content Standard E: Science and Technology

- Abilities of technological design
- Understandings about science and technology

Content Standard F: Science in Personal and Social Perspectives

- Personal and community health
- Natural resources
- Environmental quality
- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

NONPOINT SOURCE POLLUTION LESSON PLAN

Radish Seed Bioassay Worksheet

(adapted from Rathbun, 1996)

Wear protective gloves and safety glasses when handling sample solutions!

1. Soak radish seeds for 20 minutes in about 20 ml of a 10% solution of household bleach in distilled water, then rinse 5 times with distilled water. The solution kills fungi, which could interfere with seed germination.

2. Bioassays are carried out in zip-lock plastic bags containing a paper towel saturated with the test solution. Set up three replicate bags for each sample to be tested, plus three bags for each control solution. Label each bag with the sample number, plus a letter to identify the individual replicates (so for sample number 1, there would be three bags labeled "1-A," "1-B," and "1-C").

3. Pour in enough sample solution to saturate the filter paper, as directed by your teacher. Use the same volume in all tests.

4. Using forceps, place 10 seeds on the paper, spaced evenly. Seal the top of the bag.

5. Incubate the bags at room temperature for 5 days. Do not place the bags in direct sunlight! Each day, record the number of seeds that germinated in each plate, and measure (to the nearest mm) the length of the root that has emerged from each germinated seed.

6. For each sample (including the controls), calculate the mean and standard deviation of root lengths. Comparisons can be made by using the Student's t-test. (if you are not familiar with these calculation, there are many examples on the internet, such as *http://www.sonoma.edu/users/h/hanesda/B324/stats.html* or *http://www.cquest.utoronto.ca/botany/bio250y/labs/lab_notes/lab8/8tech.html*).

A more approximate method is to compare the mean ±1 standard deviation of each sample to the control. If a sample's mean plus 1 standard deviation is less that the mean of the control minus 1 standard deviation, there's a strong likelihood that the sample is **significantly more toxic than the control.**

NONPOINT SOURCE POLLUTION LESSON PLAN

NPS Subject Review: Self Test

1. Contamination of air, land, or water by substances that can adversely impact human health and the environment is known as __________.

2. Polluting substances are usually __________ materials.

3. Pollution is something in the __________ place at the _____ time in the __________ quantity. (one word)

4. Sometimes, it is not the type of material, but its __________ that determines whether or not the material is a pollutant.

5. Epidemics in the Middle Ages such as cholera and typhoid fever were directly related to __________ conditions caused by human and animal waste and garbage.

6. In 1347, a bacterium carried by rats and spread by fleas resulted in the outbreak of __________. (two words)

7. Any single identifiable source of pollution from which pollutants are discharged is known as ________________. (three words)

WORD BANK

runoff
combined sewer system
CAFOs
wrong
concentration
unsanitary
bubonic plague
point source pollution
economy
aluminum
soil erosion
pollution
waste
tillage

NPDES
test
beneficial insects
storm drains
atmospheric
acid rain
nutrients
retention ponds
constructed wetlands
porous
atmospheric deposition
fossil fuels
suspended sediment
spray drift

pathogenic
Norwalk
Mussel Watch
buffer strips
impervious
abandoned mines
eutrophication
hypoxia
algal blooms
toxins
shutoff valves
bare
bioaccumulation

8. __________ refers to storm water that flows over surfaces like driveways and lawns.

9. A __________ is a system in which waste material is mixed with urban runoff. (three words)

10. Large farms that raise many animals such as cows and chickens are known as __________. (abbreviation)

11. The Clean Water Act established the __________ under which factories, sewage treatment plants, etc. must obtain a permit to discharge wastes into any body of water. (abbreviation)

12. Most nonpoint source pollution occurs as a result of _______________.

13. Nonpoint source pollution not only affects ecosystems; it can also have harmful effects on the __________.

14. Water can run off of __________ surfaces without being absorbed.

15. In many towns and cities, the water flowing into _______ is not treated before emptying into nearby waterbodies. (two words)

16. Approximately two-thirds of the lead and mercury that enter the Great Lakes originate from __________ sources.

17. __________ is created when sulfur and nitrogen oxides react with oxygen, water, and other atmospheric compounds. (two words)

18. Prolonged exposure to acid rain can cause soils to lose important __________ such as calcium and magnesium.

19. . As acid rain flows over and through soils, it releases __________ into lakes and streams, which can cause fish to become chronically stressed.

20. Heavy machinery used by forestry and mining operations increases the risk of __________. (two words)

21. In the mid-Atlantic and Appalachian regions of the United States, acid drainage and associated contamination from __________ have caused pollution in various water bodies. (two words)

22. The primary __________ of concern in nonpoint source pollution are nitrogen and phosphorus.

23. __________ results in an overproduction of organic matter, especially algae.

24. Very low levels of oxygen in water bodies is known as __________.

25. Eutrophication may be associated with conditions that result in harmful __________. (two words)

26. When fish and shellfish feed on HABs, they can accumulate __________ that the algae produce.

27. Lawn fertilizers and pet wastes can cause pollution from excess __________.

28. Combustion of __________ is a major source of nutrients in the atmosphere. (two words)

29. Muddy water is caused by soil in the water known as __________. (two words)

30. Pesticides can enter a waterbody as __________ which occurs when wind blows sprayed pesticide into a nearby waterbody. (two words)

31. The process in which substances build up in the tissues of animals is called __________.

32. Hydrocarbons and heavy metals from automobiles and factories eventually end up in water bodies through _________ or runoff. (two words)

33. Disease-causing microbes are said to be __________.

34. The __________ virus causes intestinal illness and is transmitted to humans by food or water contaminated with feces.

35. __________ is a program designed to monitor levels of chemicals in oysters, mussels, and sediments. (two words)

36. __________ capture runoff or storm water to trap sediments and contaminants. (two words)

37. __________ slow runoff, absorb contaminants, and provide habitat for wildlife. (two words)

38. __________ paving materials allow rainwater and stormwater to drain into the ground beneath the paving. (two words)

39. __________ are strips of grass located between a farm field and a body of water. (two words)

40. Conservation __________ involves leaving some crop residue from a previous harvest while planting a new crop.

41. To ensure that nutrients are applied only as needed, farmers __________ their fields prior to the growing season.

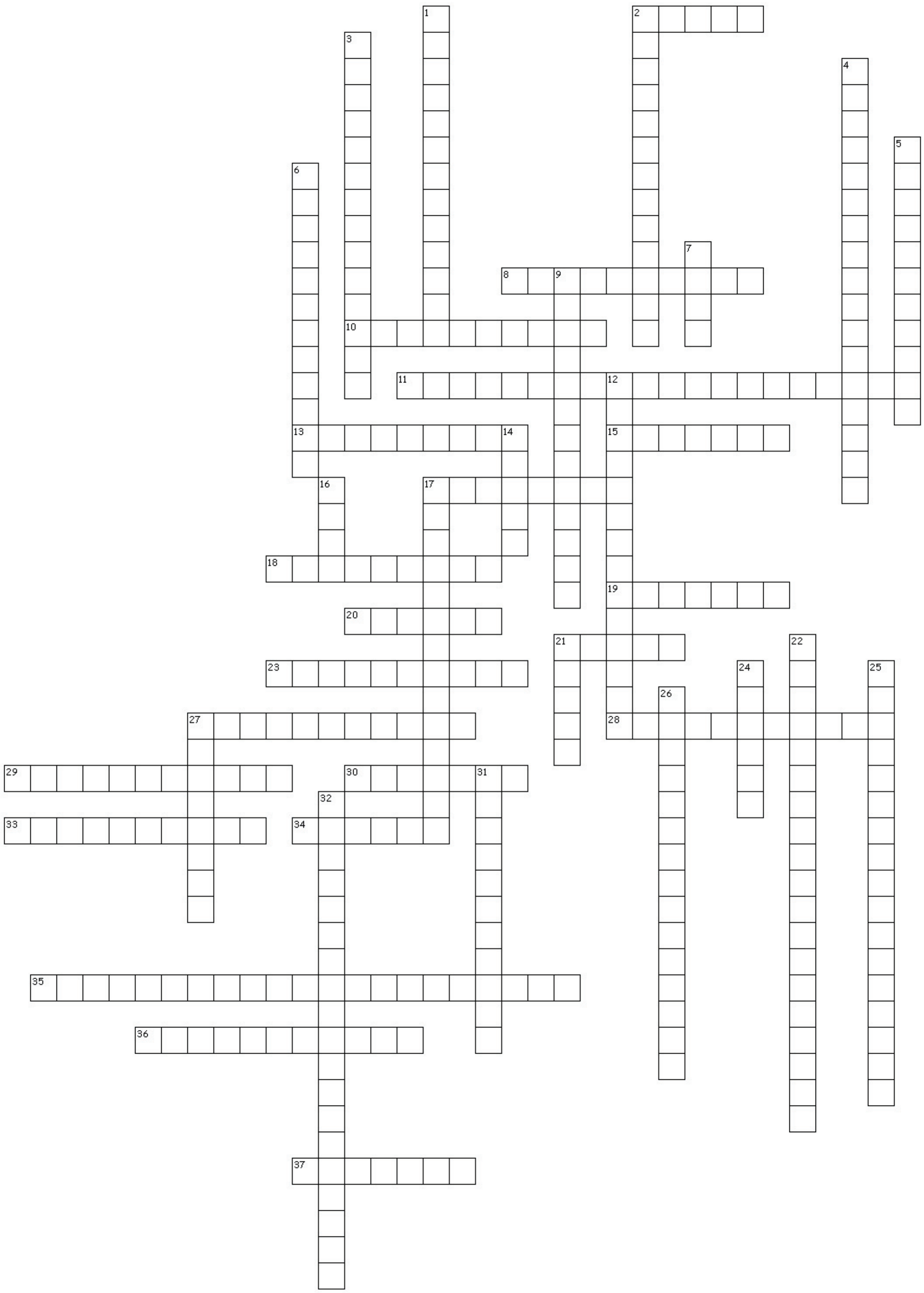
42. Lady bugs, praying mantis, and spiders are examples of __________. (two words)

43. __________ on fuel pumps on docks help limit spillage into water bodies. (two words)

44. One of the most important things you can do to reduce nonpoint source pollution is to plant grass, trees, and shrubs in __________ areas.

NONPOINT SOURCE POLLUTION LESSON PLAN

NPS Subject Review: Crossword Puzzle



Across

2. Large farms that raise many animals such as cows and chickens are known as __________. (abbreviation)
8. Epidemics in the Middle Ages such as cholera and typhoid fever were directly related to __________ conditions caused by human and animal waste and garbage.
10. Water can run off of __________ surfaces without being absorbed.
11. Any single identifiable source of pollution from which pollutants are discharged is known as __________. (three words)
13. Contamination of air, land, or water by substances that can adversely impact human health and the environment is known as __________.
15. Conservation __________ involves leaving some crop residue from a previous harvest while planting a new crop.
17. __________ is created when sulfur and nitrogen oxides react with oxygen, water, and other atmospheric compounds. (two words)
18. Prolonged exposure to acid rain can cause soils to lose important __________ such as calcium and magnesium.
19. The __________ virus causes intestinal illness and is transmitted to humans by food or water contaminated with feces.
20. __________ refers to storm water that flows over surfaces like driveways and lawns.
21. Pollution is something in the __________ place at the __________ time in the __________ quantity. (one word)
23. Disease-causing microbes are said to be __________.
27. Eutrophication may be associated with conditions that result in harmful __________. (two words)
28. Heavy machinery used by forestry and mining operations increases the risk of __________. (two words)
29. Combustion of __________ is a major source of nutrients in the atmosphere. (two words)
30. Nonpoint source pollution not only affects ecosystems; it can also have harmful effects on the __________.
33. Pesticides can enter a waterbody as __________ which occurs when wind blows sprayed pesticide into a nearby waterbody. (two words)
34. When fish and shellfish feed on HABs, they can accumulate __________ that the algae produce.

35. Hydrocarbons and heavy metals from automobiles and factories eventually end up in water bodies through _________ or runoff. (two words)
36. Approximately two-thirds of the lead and mercury that enter the Great Lakes originate from __________ sources.
37. Very low levels of oxygen in water bodies is known as __________.

Down

1. In 1347, a bacterium carried by rats and spread by fleas resulted in the outbreak of __________. (two words)
2. Sometimes, it is not the type of material, but its __________ that determines whether or not the material is a pollutant.
3. __________ results in an overproduction of organic matter, especially algae.
4. Muddy water is caused by soil in the water known as __________. (two words)
5. In many towns and cities, the water flowing into __________ is not treated before emptying into nearby waterbodies. (two words)
6. __________ are strips of grass located between a farm field and a body of water. (two words)
7. One of the most important things you can do to reduce nonpoint source pollution is to plant grass, trees, and shrubs in __________ areas.
9. __________ on fuel pumps on docks help limit spillage into water bodies. (two words)
12. __________ capture runoff or storm water to trap sediments and contaminants. (two words)
14. Clean Water Act established the __________ under which factories, sewage treatment plants, etc. must obtain a permit to discharge wastes into any body of water. (abbreviation)
16. To ensure that nutrients are applied only as needed, farmers __________ their fields prior to the growing season.
17. In the mid-Atlantic and Appalachian regions of the United States, acid drainage and associated contamination from __________ have caused pollution in various water bodies. (two words)
21. Polluting substances are usually __________ materials.
22. __________ slow runoff, absorb contaminants, and provide habitat for wildlife. (two words)

24. __________ paving materials allow rainwater and stormwater to drain into the ground beneath the paving. (two words)
25. Lady bugs, praying mantis, and spiders are examples of __________. (two words)
26. The process in which substances build up in the tissues of animals is called __________.
27. As acid rain flows over and through soils, it releases __________ into lakes and streams, which can cause fish to become chronically stressed.
31. __________ is a program designed to monitor levels of chemicals in oysters, mussels, and sediments. (two words)
32. A __________ is a system in which waste material is mixed with urban runoff. (three words)