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Fate and Environmental Impact of Pesticides in Plastic Mulch Production Runoff: Field and Laboratory Studies

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Concentrations of copper, azinphosmethyl, chlorothalonil, and endosulfan sulfate ranged from less than 1 to greater than 1000 μg/L in runoff from tomato plastic mulch production. When this runoff entered local creeks, the copper concentration was as high as 22 μg/L, which exceeded the measured larval clam LC_{50} values of 21 and 12 μg/L Cu at 96 and 192 h, respectively. A greenhouse scale investigation of copper and toxicity demonstrated that sedimentation reduced total copper concentration in runoff by 90%, although the dissolved copper concentration was unchanged, averaging 139 ± 55 μg/L. When runoff was applied to marine mesocosms containing grass shrimp and mummichog fish, unsettled runoff produced the greatest mortality, although even settled runoff caused more mortality than that in the control mesocosm receiving runoff without added copper. Desorption of soil-sorbed copper occurred quickly in saline water and contributed to toxicity. Copper toxicity in runoff can be reduced, but not eliminated, by sedimentation.

KEYWORDS: Clams; copper; plastic mulch production; runoff; sedimentation; tomato; toxicity

INTRODUCTION

Land use practices can influence adjacent water quality, particularly in coastal areas where the land−water interface is extensive, and farming, fishing, and shellfish aquaculture often share common watersheds. Since the 1970s, plastic mulch production has been used to grow tomatoes and other valuable vegetable crops such as eggplant, strawberries, green peppers, and cucumber on the Eastern Shore of Virginia. This agricultural technique uses raised planting mounds covered with 1.2−1.5 m wide, 1.25−1.50 mm thick polyethylene plastic film. Drip irrigation lines, located under the plastic on each mound, provide irrigation and fertilizer additions. This practice results in 55−75% of a typical field being covered by impermeable plastic (*1*). Characteristics of plastic mulch production include minimized herbicide use and sorption of organic pesticides, and increased disease resistance, moisture control, temperature control, and fertilizer stabilization (*2*−*4*). Nonpoint source runoff increases with plastic mulch production; the volume of pesticide-laden runoff can increase by 70% (*5*), and high concentrations of pesticides can drain into local waterways (*6*). The U.S. Department of Agriculture (USDA) reported that plastic mulch produced 4 times the runoff, 15 times the sediment load, and 19 times the pesticide load than hairy vetch mulch (*7*).

In Virginia, tomatoes are a major crop grown with plastic mulch. Crop protectants that are topically applied to tomato plants include chlorothalonil, mancozeb, maneb, metalaxyl, benomyl (*8*), and copper applied at 0.25 to 3 lb per acre per week to control bacterial and fungal diseases (*9*, *10*). Reapplication of copper is common after rain events wash the copper from the plants. Although the pesticide label cautions farmers to prevent contamination of the surrounding waterways, no specific instructions pertain to use of copper with plastic mulch production fields (*9*, *11*).

Shellfish aquaculture in coastal Virginia is dominated by hard clam (*Mercenaria mercenaria*) production and was a $6.4 million business in 1992. Shellfish are commercially grown in aquaculture facilities using water from local estuarine and tidal creeks. Many agricultural pesticides are toxic at low doses to aquatic life; this is particularly true for shellfish which are sensitive species (*12*). Copper is generally toxic to aquatic organisms, with lethal concentrations values at which 50% of the organisms die (LC_{50}) varying from 5 to 100000 μg/L (*13*, *14*). During the 1990s, the shellfish aquaculture industry on the Eastern Shore of Virginia experienced an alarming mortality to larval clams. The deaths occurred during the late spring and summer and were linked to periods of heavy rainfall. Poor water quality and possibly copper were indicated as problems. For larvae of *M. mercenaria* the 8−10 day LC_{50} is 16.4 μg/L Cu from added copper chloride (*15*). To protect aquatic life in Virginia, the saltwater quality criteria are 2.9 μg/L dissolved copper both as a 1-h concentration not to be exceeded more than once every three years, and as a four-day average not to be exceeded more than once every three years (*16*).

The specific objectives of this research were the following: (1) to evaluate the concentrations of copper-based pesticides and organic pesticides in runoff from tomatoes grown with plastic mulch production; (2) to evaluate the loading of copper to waterways adjacent to the tomato crop grown with plastic mulch production; (3) to evaluate potential toxic impacts of the

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copper concentrations to larval clams; and (4) to investigate the effectiveness of sedimentation as a best management practice for reducing copper and toxicity from plastic mulch runoff.

MATERIALS AND METHODS

Laboratory Chemicals and Analyses. All chemicals, polyethylene sample containers, and glassware were purchased from Fisher Scientific (Raleigh, NC). All plasticware and glassware were soaked in 10% trace metal grade nitric acid for 8−12 h, rinsed with distilled water three times, then rinsed with Nanopure purified water three times. All aqueous samples for copper determination were collected in acid-washed high-density polyethylene containers. Samples for organics analyses were obtained in clean, amber glass, 1-L bottles.

Salinity was determined by a hydrometer; pH was measured by a Fisher model 620 Accumet meter or colorimetric paper. Dissolved oxygen was measured with a YSI model 58 dissolved oxygen meter that compensated for salinity. Total suspended solids were determined according to Standard Method 2540D (*17*).

Dissolved copper was determined according to EPA Method 3010 (*18*), using 0.45-μm filters (Gelman Sciences) when the sample pH was below 8. Samples with pH >8 were centrifuged at 14000 rpm for 30 min because 0.45-μm membrane filters sorb dissolved copper at pH >8 (*19*). Total copper was digested according to EPA Method 3020A (*18*). Concentrations below 50 μg/L Cu were measured according to EPA Method 7211 using a Perkin-Elmer 5100PC graphite furnace atomic absorption spectrophotometer (*18*) with a detection limit of 0.5 μg/L Cu. For higher concentrations, a Perkin-Elmer 703 flame atomic absorption spectrophotometer was used following manufacturer's recommendations and EPA Method 7210 (*18*). Organic pesticides were extracted from whole water samples (water plus particles) with dichloromethane using either EPA Method 625 (*20*) or EPA Method 608 (*20*), and the extracts were analyzed using gas chromatography/mass spectrometry with a Hewlett-Packard HP5980a/HP5970 system with a 30-m, 0.25-mm i.d., HP Ultra II column. Organic pesticides were identified on the basis of their mass spectra and then confirmed with standards. Quality control samples included travel blanks, analysis blanks, and analysis spikes. All statistical analyses were performed with NCSS 97: Statistical System for Windows (Kayesville, UT; 1997).

Field Sites and Investigation. The 1996−1998 sampling program assessed copper levels in runoff and water samples from creeks at the Eastern Shore of Virginia with and without plastic mulch production of tomatoes in their watersheds. The control watershed was Raccoon Creek, which has contained no agricultural activity since 1940 and is an isolated estuarine tidal creek that drains the Eastern Shore National Wildlife Refuge in Cape Charles, VA. Parker's Creek is a nontidal freshwater creek adjacent to plastic mulch fields containing tomatoes. Gargathy Creek is a tidal creek that drained a rural watershed composed of greater than 55% agricultural land with 7−9% of this cropland in plastic mulch production; no marinas and little boat traffic were present. Gargathy Creek was impacted by runoff from five or six tomato plastic mulch fields during 1996−1998.

Aqueous grab samples for salinity, suspended solids, pH, and copper were collected at low tide. When sufficient runoff occurred to cause drainage from the land, grab samples were taken at the edges of fields, roadside ditches, and local low spots for determination of copper and organic pesticides. Time programmable autosamplers (ISCO model 2700, ISCO, Inc.; Lincoln, NE) were deployed during periods of rainfall and no rain in the Raccoon, Parkers, and Gargathy Creeks. The programmable autosamplers had poly(tetrafluoroethylene) tubing and polyethylene bottles and collected water samples at 2-h intervals; copper, salinity, and TSS were determined in these samples.

***M. mercenaria* Toxicity.** Artificial seawater was prepared by adding Instant Ocean to Nanopure purified water. Artificial seawater was processed through a 35-μm screen, sterile 0.2-μm filter, and 0.45-μm filter directly prior to use. Dissolved and total copper measurements for 26.5 ppt artificial seawater ranged between 1 and 2 ppb. FisherBrand copper nitrate standard was diluted with artificial seawater to prepare toxicant solutions at desired concentrations. All toxicant solutions had initial dissolved oxygen concentrations of ≥ 7 mg/L and pH values between 8 and 8.5, and they were maintained at 24 °C so as to be in the range of ideal conditions for *M. mercenaria* development (*21*).

Isocrysis galbana (parke.) (Carolina Biological Supply, Burlington, NC) were fed Florida Aquafarms Microalgae Grow; the algal culture was maintained at 26.5 ppt artificial seawater at 24 °C without aeration. A light cycle of 16 h on and 8 h off from cool white fluorescent bulbs, ∼230 lx, was used for established cultures. Algae were concentrated by centrifugation at 3500 rpm for 20 min.

Prodissoconch (straight hinge) *M. mercenaria* in 13 °C natural seawater were obtained from an Atlantic coast hatchery. Once received at the laboratory, clams were acclimated over a 24-h period by siphon addition of *I. galbana* at 100000 cells/mL in 26.5 ppt artificial seawater. The clams were fed daily to maintain flask concentrations of 100000 algae cells/mL and artificial seawater changes were made every 48 h.

Three replicates of 10 ± 1 7-day old larval clams (*M. mercenaria*) were treated at each of the following doses: 5, 7, 14, 29, 57, 119, 240, and 495 μg/L dissolved Cu. Five replicate controls were prepared with background copper content of 1 μg/L. Clam larvae in 1.5 mL of toxicant solution with 100000 cell/mL *I. galbana* were contained in clear 30-mm plastic Petri dishes. The dish was sealed with petroleum jelly applied to the interior perimeter of the Petri dish top. This minimized evaporation to $<3.5\%$ through 288 h (3.5% evaporative loss as equivalent to 1 ppt salinity increase). Using either an Olympus CH-2 light microscope or Bausch and Lomb WP 7854 stereoscope, daily observations of live and dead larvae were made. LC_{50} values were calculated for algae-fed larval clams exposed to copper using the U.S. EPA Trimmed Spearman Karber software (*22*).

To test the impact of copper source, two replicates of 7-day-old larval clams were treated at 8 and 15 μg/L total Cu with either copper nitrate or Kocide 101 (Griffin Chemical Co., Valdosta, GA). Four controls were also prepared and the toxicity tests were conducted as described above.

Sedimentation and Mesocosm Studies. A detailed description of the greenhouse, the simulated tomato fields, addition of copper-based pesticides, rainfall events, soil, runoff, and water sampling is presented elsewhere (*23*).

Bojac sandy loam topsoil was obtained from the Eastern Shore of Virginia. The soil contained a total copper content of 4.25 ± 0.18 mg/kg of soil, 1.2 ± 0.06% organic matter, and 0.8 ± 0 mg/L plant-available copper. Simulated tomato fields, or 2.1 m × 1.2 m × 0.9 m soil bins containing about 1.5 tons of soil, were constructed to simulate three conditions: (1) typical plastic mulch field; (2) plastic mulch field with sedimentation control; (3) a control field without plastic mulch or copper additions. The soil was shaped into two 20-cm high mounds; ports for collecting runoff and leachate were installed. Patio hybrid tomatoes were planted and successfully grown in two lengthwise rows for a total of fourteen plants per bin. A rainfall simulator applied 54 L of distilled water to each field during each of the nine rainfall events over the summer growing season. For the treated soil bins, Microsperse copper crop protectant (Microflow, Inc., FL) was regularly applied eleven times by spray applicator at a target concentration of 1190 mg/L free copper. The total amount of copper added was 9.9 g to each treated field, with a mean of 0.9 g/application and a range of 0.6−1.5 g/application.

At the end of the tomato-growing season, desorption of copper bound to the Bojac sandy loam was investigated using soil samples taken from the exposed regions of the simulated tomato fields. The measured copper concentration in subsamples of the soil ranged from 20 to 25 mg of Cu/kg of soil. Varying amounts (0, 10, 25, 50, or 75 g) of well-mixed, copper-laden soil were added to 225 mL of copper free solution in 250-mL plastic bottles. Both 0 and 20 ppt artificial seawater in Nanopure water were tested in triplicate. The bottles were placed on a rotary end-over-end shaker at 30 rpm. Samples of 15 mL each were taken at 30 min, 2 h, and 24 h; total and dissolved copper were determined.

Runoff and leachate from the soil bins were collected during the rainfall events. All runoff and leachate samples were analyzed for total suspended solids as well as total and dissolved copper. The composite runoff from the simulated plastic mulch field with sedimentation control

Table 1. Concentrations of Organic Pesticides and Copper Detected in Tomato Field Runoff and Rain Puddles in Nonagricultural Areas during 1996–1998 Growing Seasons

		pesticide concentration, μg/L[a]				
no.	sample type	dissolved copper	azinphos-methyl	chloro-thalonil	endosulfan sulfate	total copper
1	tomato field 1 runoff	–	nd	80	nd	210
2	tomato field 2 runoff	–	nd	2.1	0.6	68
3	tomato field 3 runoff	–	264	165	2.4	1410
4	tomato field 4 runoff	–	177	64	1.7	412
5	tomato field 5 runoff	–	nd	0.16	0.02	21
6	tomato field 6 runoff	208	–	–	–	–
7	tomato field 7 runoff	108	–	–	–	–
8	tomato field 8 runoff	236	–	–	–	–
9	tomato field 8 runoff	35	–	–	–	–
10	tomato field 9 runoff	20	–	–	–	–
11	rain puddle 1[b]	–	nd	nd	nd	5
12	rain puddle 2	–	nd	nd	nd	12
13	rain puddle 3	–	nd	nd	nd	8
14	field blank	–	nd	nd	nd	nd

[a] nd = not detected; – = not analyzed. [b] Rain puddles were located in either residential areas or in a wildlife refuge.

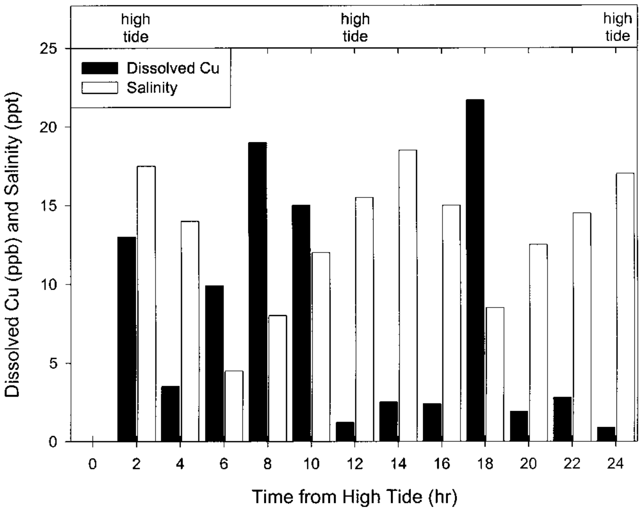

Figure 1. Salinity and copper concentrations in Gargathy Creek during a 2.5-in. rainstorm on September 4, 1998. Runoff from five tomato fields entered the creek.

was held in a 5-gal plastic bucket where it settled for 5 days. After settling, the clarified water was carefully decanted. Runoff and leachate from each of the three fields were then added to marine mesocosms. There were three separate marine mesocosms; each received runoff and leachate from one of the tomato field simulations: (1) typical plastic mulch field; (2) plastic mulch field with sedimentation control; (3) control field. The mesocosms were dosed eight times with runoff from the simulated tomato fields.

A marine mesocosm, as described in ref *24*, consisted of three subunits, each constructed of three 0.6 m × 0.9 m × 1.2 m glass aquariums, serially linked by 7.6-cm i.d. conduits that allowed water and mobile organisms to move freely between units. Before filling the mesocosm, six 0.3 m × 0.3 m × 0.3 m plexiglass boxes were placed on pylons in each of the three aquariums. The boxes were set at differing heights to represent high, mid, and low intertidal zones; a 250-mm mesh netting was placed at the faces of the plexiglass boxes to allow the flow-through of water. The mesocosms were filled with samples of marsh sediment, plants, and animals obtained from a historically clean reference marsh on the southeastern coast of the United States. The plants were *Spartina alternaflora* in low and mid marsh samples and *Salicornia virginica* in high marsh areas. Pumps attached to the mesocosms circulated 100 gal of water to simulate tidal cycles. Each mesocosm contained 100 gal of 20 ppt artificial seawater and was monitored daily for pH, temperature, and salinity. Salinity was maintained at 20 ppt by adding Instant Ocean as necessary. Runoff and leachate doses were added at low tide.

Organisms for the toxicity tests were collected from tidal channels by seine. Ten grass shrimp (*Palaemonetes pugio*) and 10 fish (mummichogs, or *Fundulus heteroclitus*) were placed in each of the three subunits of each mesocosm shortly before dosing runoff and leachate. The organisms were contained in multiple clear plastic 200-mL wide-mouth containers with a layer of mesh for a lid to allow the bottles to circulate mesocosm water. Mortality was monitored and recorded every 24 h over a period of 96 h.

Samples were collected from the estuaries immediately before and after the addition of the runoff and leachate from the simulated agricultural fields. ISCO autosamplers were used to take samples from the mesocosms every 4 h for 48 h following the addition of the runoff and leachate. All samples were analyzed for pH, salinity, suspended solids, dissolved copper, and total copper.

RESULTS

Field Studies. Table 1 presents data for copper and organic pesticides measured in actual runoff (samples of water plus particles) from tomato plastic mulch production. That the concentration values appear highly variable is a reflection of the composite factors that affect pesticide concentrations in runoff. These factors include amount of rainfall, amount of pesticide applied, and length of time between rainfall and pesticide application. Samples could not be collected from conventional agricultural fields (fields that did not have plastic mulch) because there was no runoff from these field. The sandy-loam soils of Virginia's eastern shore are very permeable, and when no plastic mulch is present the rain readily infiltrates into the soil.

Dissolved copper levels were extremely high (up to 236 μg/L) in some samples. When both organic pesticides and copper were determined, samples containing copper frequently contained detectable or even high concentrations of azinphos-methyl, chlorothalonil, or endosulfan, all of which are used on tomato crops. The runoff represented by these samples drained into either Gargathy Creek or Parker's Creek.

The copper contaminated runoff measured in samples 6−10 of **Table 1** entered Gargathy Creek on September 4, 1998, causing the creek's concentration to reach as high as 20 μg/L during low tide (**Figure 1**). The copper concentrations in Gargathy Creek during runoff events fluctuated with the tide, and the lowest concentrations were observed at high tide when the dilution was the greatest. The highest dissolved copper concentrations were observed during the low tides when runoff was less diluted.

Figure 2 illustrates the different time pattern of total suspended solids and dissolved copper in nontidal Parker's Creek. Samples collected before a rainstorm indicated low suspended solids and low dissolved copper concentrations in the creek prior to commencement of the rain and runoff. A large spike in both TSS and dissolved copper concentrations was seen in the creek when a first flush of runoff entered. The concentrations declined with time due to cessation of both rain and runoff coupled with copper and sediment transport.

Copper concentrations in Raccoon Creek, the control watershed, were monitored during periods of rainfall and no rain throughout 1996−1998. For 13 months in which data were collected, monthly averages for dissolved copper concentration in Raccoon Creek were at or below 4 μg/L Cu. No significant difference was determined for copper concentration during periods of rain and no rain.

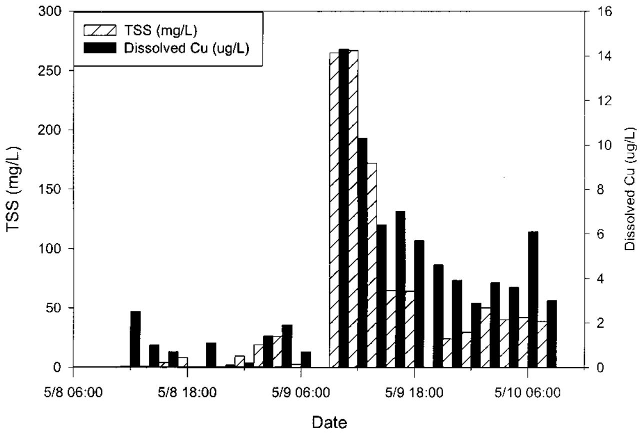

Figure 2. Total suspended solids (TSS) and dissolved copper concentrations in Parker's Creek before and during a storm event in the 1998 growing season; storm began at 8 a.m. on May 8, 1998.

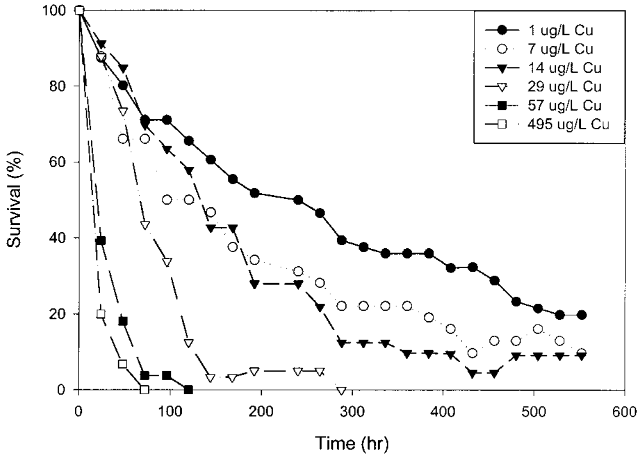

Figure 3. Survival of larval clams at varying copper concentrations.

***M. mercenaria* Toxicity. Figure 3** presents selected results of the copper toxicity to larval clams. The results for the 119 and 240 μg/L treatments were similar to those of the 57 and 495 μg/L treatments. As expected, higher copper concentrations increased clam mortality at a given exposure time. In the four highest copper doses, 100% mortality to the larval clams was observed within 120 h. The mortality data at all concentrations between 1 and 495 μg/L were used to calculate the lethal concentration at which 50% of the organisms die (LC_{50}) for algae-fed larval clams exposed to copper. Calculated LC_{50} values were 142, 62, 21, and 12 μg/L Cu at 24, 48, 96, and 192 h, respectively. Using the analysis of variance function ($\alpha = 0.05$, one-sided test with Bonferroni adjustment) in the U.S. EPA Trimmed Spearman Karber software for percent survival data at 288 h, a lowest observed adverse effect level of 14 μg/L Cu and a no observed adverse effect level of 7 μg/L were calculated for mortality.

No difference in toxicity response for larval clams was observed between Kocide 101 and copper nitrate. The inert ingredients in the Kocide 101 formulation did not appear to have major ameliorating or synergistic effects on clams at these concentrations.

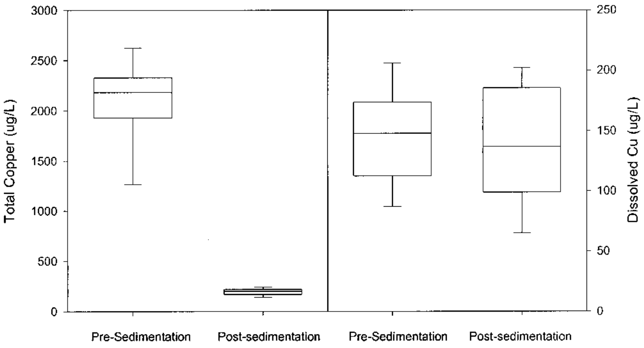

Figure 4. Comparison of total (left) and dissolved (right) copper concentrations before and after sedimentation. Data are shown for simulated plastic mulch field with sedimentation.

Sedimentation and Mesocosm Studies. On the basis of mass balance calculations of the total amount of applied copper and the amount leaving the soil bin, nearly all, or 99%, of the applied copper mass remained on the simulated tomato field. The top 2.5 cm of soil in the middle of the soil bin that was not covered with plastic mulch sorbed the most copper. Although the initial Bojac sandy loam contained only 4.25 ± 0.18 mg of Cu/kg of soil, analysis of the soil cores showed that for both simulated plastic mulch fields, the copper levels in the top 2.5 cm were 26 ± 18 mg of Cu/kg of soil along the region of exposed soil between the plastic covered mounds. The control field, which received no copper applications, had a mean copper content of 2.65 ± 0.25 mg of Cu/kg of soil throughout the field surface and depth. This mean is lower than the original copper concentration measured for the soil, indicating that copper was leached by the nine rain events that occurred over the course of the study.

Only 1% of the applied copper mass left the copper-treated simulated tomato fields. Of that, 82% of the copper was found in the runoff, and was composed of 74% sorbed to the suspended solids and 8% dissolved in the runoff. For the 18% of the copper that was found in the leachate, 10% was dissolved and 8% was sorbed to suspended particles. The concentrations of copper in the runoff and leachate were relatively high, despite representing only 1% of the applied copper mass (9.9 g of copper per treated field). Before sedimentation, there was no significant difference in the total and dissolved copper concentrations from the two simulated plastic mulch fields. The volume of plastic mulch runoff varied from 9 to 13 L per rain event and averaged 2102 ± 433 μg/L total copper and 189 ± 139 μg/L dissolved copper. The volume of leachate per rain event ranged from 9 to 17 L and averaged 312 ± 198 μg/L total copper and 216 ± 99 μg/L dissolved copper. As shown in **Figure 4**, sedimentation treatment reduced the total copper concentrations by 90% to a final concentration of 245 ± 127 μg/L; however, dissolved copper concentrations remained stable before and after sedimentation, averaging 139 ± 55 μg/L. Thus, implementing sedimentation can significantly reduce, but not eliminate, inputs of copper from runoff entering receiving streams.

The control tomato field did not receive copper-based pesticides. The dissolved copper concentrations in the runoff from the control field ranged from 6 to 31 μg/L and in the leachate ranged from 8 to 20 μg/L Cu. This is further evidence that the rainwater leached copper from the Bojac sandy loam.

Because the majority of the copper remaining on or leaving the simulated tomato fields was sorbed to the soil particles, desorption of copper from Bojac sandy loam to water was investigated. Soil was removed from copper-treated simulated tomato fields and added to 0 or 20 ppt artificial seawater. The results, presented in **Figure 5**, demonstrate that copper desorbed quickly with little difference between 30-min and 24-h sampling times. Copper desorption was similar at 0 ppt salinity water.

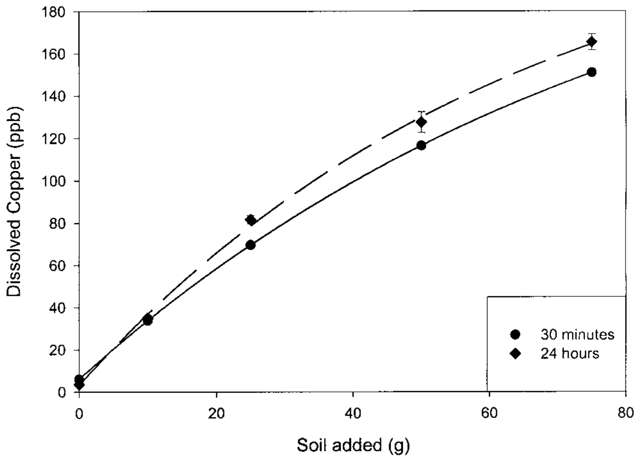

Figure 5. Desorption of copper from copper-laden Bojac Sandy Loam into 20 ppt artificial seawater. Error bars indicate one standard error.

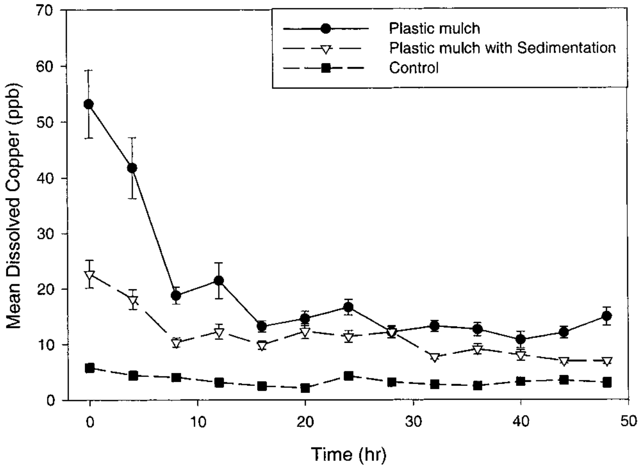

Figure 6. Copper concentrations measured in mesocosm water after the addition of runoff and leachate from the rain events on the simulated tomato fields. Each data point represents the mean value for eight trials; error bars represent one standard error.

Dissolved copper concentrations in the mesocosms increased after addition of runoff and leachate at low tide (**Figure 6**). Typically, ~24 L of water was added to a mesocosm per rain/runoff event. The dissolved copper concentration in the mesocosm receiving the unsettled runoff and leachate from the plastic mulch field was the highest, reaching nearly 55 μg/L Cu, then declining over 48 h to ~10 μg/L Cu as the tide cycled and the mesocosm sediments sorbed copper. The mesocosm receiving runoff and leachate from the plastic mulch field with sedimentation demonstrated a pattern similar to that of the mesocosm receiving runoff without sedimentation, but the highest copper concentration was only ~22 μg/L Cu, which then declined to ~8 μg/L in 48 h. The mesocosm receiving water from the control field fluctuated, without any pattern, between 2 and 6 μg/L Cu, which were much lower copper concentrations than found in either of the mesocosms to which copper-containing runoff had been added.

Survivorship data in **Figure 7** indicate that the higher copper concentrations were toxic and that sedimentation reduced toxicity. **Figure 8** presents the copper concentrations during the time that the fish and shrimp were actually present in the mesocosms. Minimal mortality was observed in the control mesocosm. At 48 h, fish mortality at or near 50% was measured for the mesocosm associated with the unsettled runoff. That same level of mortality did not occur until after 96 h in the mesocosm receiving runoff with sedimentation. The fish in the mesocosm receiving runoff after sedimentation survived as well as the controls for up to 72 h, when only 1 of 30 fish died.

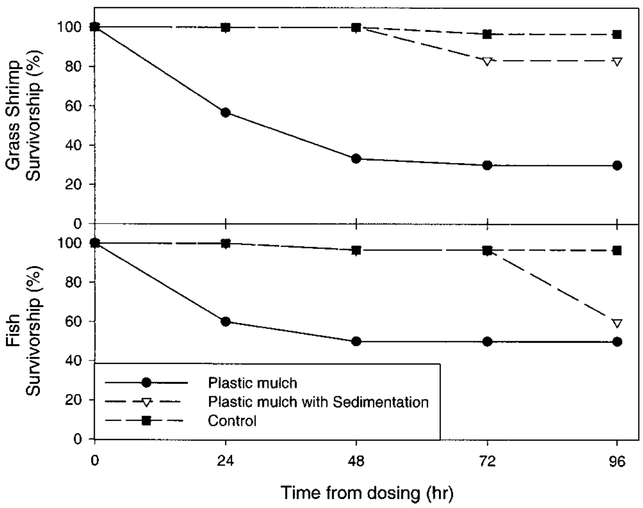

Figure 7. Survival of grass shrimp (*P. pugio*) and mummichog fish (*F. heteroclitus*) vs time in mesocosms receiving runoff and leachate from simulated tomato fields.

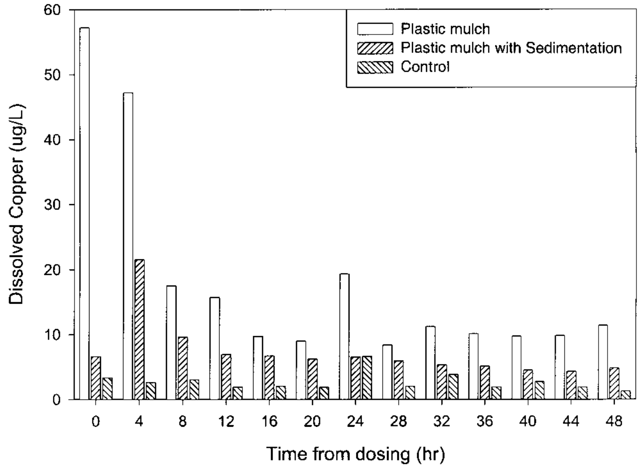

Figure 8. Dissolved copper in mesocosms during the time that the fish and shrimp were present. Low tide occurred at 0, 12, 24, and 36 h.

The shrimp mortality was consistent with that of the fish (**Figure 7**). Mortality in the first 48 h was in the range of 70% for the mesocosm receiving inputs from the plastic mulch field without sedimentation, whereas no mortality occurred within 48 h in the mesocosms associated with the control field or the field with sedimentation. At 96 h, the mortality due to inputs from the plastic mulch field with sedimentation was only 16%, which is less than one-fourth of the mortality in the nonsettled mesocosm. The copper concentration in the mesocosm associated with sedimentation had about half the copper concentration of the mesocosm without sedimentation (**Figure 8**); at the lower concentrations of copper, a longer exposure time was required for mortality to occur. The water volumes and dissolved copper levels in the runoff and leachate were similar for the two plastic mulch fields, although the total copper concentration was a factor of 10 higher for the nonsettled runoff. Therefore, the higher dissolved copper concentration in the mesocosm associated with no sedimentation was due to desorption of copper sorbed to the soil. The desorption was fast, as shown by the high copper concentration measured in the mesocosm with the first sample.

DISCUSSION

During rainfall events on the eastern shore of Virginia, runoff usually only occurred from plastic mulch fields because the rainwater infiltrated into the soil at fields planted using conventional agriculture (e.g., corn, soybeans, and cotton) or forested lands. Other researchers have also noted increased runoff from plastic mulch fields as compared to that of conventional fields (*5*). The concentrations for copper, azinphosmethyl, endosulfan, and chlorothalonil in full-scale tomato plastic mulch runoff indicated that concentrations were as high as hundreds of parts per billion. These concentrations were consistent with those measured by other researchers (*25*) for plastic mulch runoff from tomato fields. Although high concentrations were diluted when the runoff drained into receiving streams, measured concentrations of copper in Gargathy Creek and Parker's Creek were still in the tens of parts per billion and exceeded reported values for toxicity of aquatic organisms that inhabit these waterways.

The focus of this research was possible impacts of runoff to the commercial clam industry. The 22 μg/L dissolved copper concentration measured in Gargathy Creek exceeded the reported LC_{50} for larval clams of 11.4−16.4 μg/L added copper for an 8−10 day exposure (*15*), and Virginia's water quality standard of 2.9 μg/L (*16*). The extent of dilution of runoff is variable, and it depends on many factors including the amount of rainfall, the amount of runoff, the interval between pesticide applications and rainfall, the water flow in the creek, and the tidal cycle.

For a nontidal creek such as Parker's Creek (**Figure 2**), the runoff entering the creek caused a spike in copper and suspended solids concentrations which then declined with time as the rainfall ceased, and the easily removed soil particles and copper were washed off the agricultural field. A different pattern emerged for tidal creeks such as Gargathy (**Figure 1**). The pesticide input from runoff was diluted at high tide when non-pesticide-containing seawater entered the creek. This is the reason for the inverse relationship between high pesticide concentration and low tide. The flow reversal during tidal cycles transported the pesticides back into the surrounding marshland. Even after runoff ceased, these temporarily stored pesticides drained back into the tidal creek again during low tide. It generally takes about four tidal cycles for the runoff-input pesticides to be carried out of the tidal creek and into the ocean (*6*).

This research demonstrated that only 1% of the applied copper was lost from the plastic mulch fields. This percent loss was consistent with those of other highly sorbed pesticides (*26*). The majority of the copper applied to agricultural fields sorbed to the soil. As soil particles washed off the surface in runoff, they carried sorbed copper that was desorbed when the particles contacted uncontaminated water. Copper-laden Bojac sandy loam readily released copper to water of 0 ppt salinity (analogous to rainwater and runoff) and 20 ppt salinity (analogous to estuarine water) (**Figure 4**). The amount of copper released was very similar at the 30-min and 24-h time periods, indicating nearly instantaneous release. This quick release of copper from soil has been observed by other coastal researchers (*27*, *28*). Its importance is that particulate bound copper in runoff desorbs and becomes soluble copper in adjacent waterways. Over time, reequilibration with non-copper-containing soils and sediments in the water eventually will lower the dissolved concentrations. In the mesocosm studies, even though both the settled and nonsettled copper-runoff treatments contained approximately the same dissolved copper concentration, the dissolved copper concentration in the mesocosm receiving nonsettled runoff was initially higher because of desorption. Similarly, the field research for this project found a superimposable pattern for high suspended solids and dissolved copper when tomato field runoff entered Parker's Creek (**Figure 2**). In a related field study, researchers found that dissolved copper continued to be leached to runoff by rain throughout the fall, winter, and spring following application of copper to the summer tomato crop (*29*).

This research investigated the copper toxicity to larval clams. The LC_{50} values determined for larval clams were 21 and 12 μg/L Cu at 96 and 192 h, respectively. These LC_{50} values are similar to those reported by Calabrese et al. (*15*). The LC_{50} copper levels were comparable to copper concentrations detected in Gargathy Creek during storm events, a creek whose ecology supports clams and whose waters are used for commercial shellfish aquaculture.

The comparison of copper toxicity from copper nitrate and the copper pesticide Kocide 101, which contains additives and surfactants not present in copper nitrate, indicated that both forms of copper had similar toxic effects to larval clams.

The mesocosm studies demonstrated that copper from runoff definitely caused toxicity to estuarine organisms under conditions that simulated field scale conditions (**Figures 7** and **8**). The organisms studied were the grass shrimp (*P. pugio*) and the mummichog fish (*F. herteroclitus*), both of which are omnivores that consume detritus, algae, higher plants, and animals that are smaller than themselves. Thus, these organisms would be exposed to both dissolved copper and copper sorbed to sediment particles or edible particles that were ingested as food. It is well accepted that many algae accumulate copper at concentrations in excess of aquatic equilibrium (*30*) and that estuarine bivalves experience metal toxicity through particulate exposures (including algae) (*31*, *32*).

The mortality to grass shrimp and fish exceeded that of the controls when copper from runoff was present in saline water. During the time that the grass shrimp and fish were deployed to the mesocosm receiving nonsettled runoff, the copper concentration was >40 μg/L for the first 8 h, then declined to 9−20 μg/L Cu for the remaining 40 h, depending on the tidal cycle. Death to the test organisms occurred within 24 h for the nonsettled runoff. Sedimentation removed the copper bound on sediment particles, which was ∼90% of the total copper present in the runoff. This substantially reduced, but did not eliminate, toxicity. Mortality of the test organisms when sedimentation was implemented was the same as for the control mesocosm for at least 48 h, but was greater than that for the controls by 96 h. Full-scale agricultural runoff, when controlled with best management practices, has been shown to reduce mortality to grass shrimp and mummichogs in the natural estuarine environment (*33*).

There is a trend toward considering only dissolved copper for aquatic toxicity and in regulations (*34*). Many studies have reported on the copper speciation and bioavailability or toxicity (*35*−*39*) with some studies suggesting that only bioavailable copper should be considered as a source of toxicity (*39*). This research demonstrated that some portion of sediment-bound copper must also be considered toxic when evaluating loadings of agricultural copper to receiving waters.

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