Testing for Persistent Herbicides in Compost

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Composting is a sustainable practice to turn organic waste into a soil amender that promotes plant growth and quality. Organic waste consists of yard trimmings, leaves, and food waste. The composting process has been proven to help reduce herbicide residues to a low enough concentration that it is harmless to plants. However, some herbicides have half-lives that out-live the time that it takes for organic waste to break down in the composting process. These herbicides are defined as 'Persistent Herbicides' and they effect plants at low concentrations. Persistent herbicides can affect plants at a concentration of 1 ppb (The United States Composting Council, 2015). For this research, a bioassay kit provided by the Compost Research and Education Foundation was used to test compost from the Knox Farm, a home compost batch, and two different batches from Better Earth Composting–an Industrial Compost company close to Galesburg. The target audience for this research is mainly compost consumers and producers of compost, both on the large scale and small scale level.

Figure 1: *Picture of plants on April 10, 2023. This was on the 12th day of the 15 day observation period.*

Unfortunately, there are no USCC/STA-approved bioassay methods to test for these herbicides in compost, but CREF is attempting to develop a simple and quick method to test for persistent herbicides. Dr. Frederick Michel and Daisy D'angelo put together a "Persistent Herbicide Workshop" and provided the participants with a kit with all the materials except for additional composts. The persistent herbicide that was tested for was Clopyralid. Clopyralid is an active ingredient that is found in over thirty different herbicides and is used on pastures and lawns making the possibility of it being in one of the feedstocks for a compost batch more prominent. A few other herbicides that are commonly sold are Aminocylopyrachlor, Picloram, and Aminopyralid. Dow AgroSciences has developed the majority of the herbicides listed. The significance of testing for herbicides in compost is important because herbicides affect the overall health of the plant.

There is not just one definition of compost, but for the purpose of this study this definition is the most helpful. The definition is provided by Bernal et al. in the study they published titled *Current Approaches and Future Trends in Compost Quality Criteria for Agronomic, Environmental, and Human Health Benefits*. “Compost consists of a spontaneous biological decomposition of solid organic material in a predominantly aerobic environment, during which primarily bacteria, fungi, and other microorganisms transform organic materials into a stable, usable organic substrate called compost” (Bernal et al., 2017). Compost is made up of “greens” and “browns”. The greens are nitrogen-rich materials such as raw vegetables and food scraps, grass clippings, green leaves, and coffee grounds. Browns are carbon-rich materials such as fall leaves, twigs, shredded newspaper, straws, wood chips, and shredded wood. The pile also needs water and it has to be porous enough to allow air to flow through the pile (ILSR). The compost pile can heat up to 140 degrees to 160 degrees Fahrenheit during the thermophilic stage of the composting process which breaks down herbicides (ILSR). There are a few ways that an herbicide or pesticide can be broken down in compost; adsorption, volatilization, or mineralization.

Adsorption is when the chemicals break down and form bonds with other compounds.*Volatilization* is when the molecules escape into the atmosphere, “which may be accelerated by the high composting temperatures”(Michel & Doohan, n.d.). Some molecules leach from the pile with liquid run-off. . According to a Fact Sheet published by Frederick C. Michel Jr. and Douglas Doohan, *mineralization* is the most “desirable fate” for pesticides. This process is when the pesticide or herbicide is broken down completely into carbon dioxide. They also indicate that “ pesticide residues are incorporated into the organic matter fraction of compost after biotransformation in a form that is chemically different from the parent compound and therefore not biologically active” (Michel & Doohan, n.d.). Unfortunately, there are persistent herbicides that have an effect on plant health at low concentrations. Clopyralid is one such herbicide that has been a major concern because it has been shown to contaminate compost.

Herbicide Effects on Compost

There are multiple articles and studies about the effects of herbicides on compost. These studies discuss Clopyralid and other persistent herbicides that should be taken into consideration when testing for herbicides in compost. Herbicides first started becoming a topic of concern when Washington State ran tests on the eastern side of Washington because of the heavy use of herbicides on the land. Since then, different studies have used information and techniques from the study in Washington.

Clopyralid is an herbicide produced by Dow AgroSciences and is sold under the trade names: Reclaim, Stinger, Transline, Confront, Lontrel, Curtail, and Millenium Ultra (Michel &

Doohan, n.d.). It is used to control broadleaf weeds, pastures, lawns, non-cropland areas, and right-of-ways (Michel & Doohan, n.d.). This herbicide is not affected by the composting process, and most plants are not damaged by Clopyralid. However, the bean family, potato/tomato family, and the sunflower family are very sensitive to it and this herbicide can stunt plant growth at a concentration as low as 10 parts per billion. “Recent Laboratory trials have shown that clopyralid dissipated during composting with a half-life of 9-102 days, similar to soil dissipation results. However, composts may be considered market ready and distributed between 30-300 days"(Brinton et al., 2006). The normal concentration of Clopyralid that is being sprayed onto grasses and pastures on the day of application is 10,000 to 50,000 ppb (Michel & Doohan, n.d.). One concern that was raised by Michel and Doohan is that organic farm owners are worried about compost and manure contamination. Since they rely on these two materials for fertilizer and soil amendments, the possibility of herbicide contamination could get their organic certification taken away and the well-being of their crops would be affected. An article published by Biocycle in 2002 was one of the first articles that discussed the rising concerns of clopyralid and the negative effects it has on plants.

This problem became a big concern in Washington state, so Washington State University and the Washington State Department of Agriculture collected compost samples from nine major facilities, five from western Washington and four from eastern Washington (Rynk, 2002). Their results showed that clopyralid was detected at levels higher than the critical levels at every facility. “The most alarming numbers came from an eastern Washington facility where clopyralid was found in grass clippings at 1,550 ppb and at 477 ppb in immature compost” (Rynk, 2002). W.F. Brinton, E. Evans, and T.C. Blewett wrote the “Reliability of Bioassay Tests to Indicate Herbicide Residues in Compost of Varying Salinity and Herbicide Levels”, a study on different bioassay methods to try to determine the most useful and producible bioassay for the detection of herbicides in compost. A more recent study conducted in 2016 did a bioassay on Red Clover and Fava Beans planted in different samples of compost. One compost was "finished" compost, the second r was "feedstock", and the last sample was compost amended with high-carbon wood ash. Between one and eighteen composite samples were analyzed during the course of the study and the results showed that the Red Clover planted in the "finished" compost showed symptoms/ injury ratings greater than or equal to the injury ratings observed for the respective positive controls for each of the eight plantings during the study (Coker et al., 2016). An article written a year earlier by one of the authors of Coker et al article, wrote "Coping with Persistent Herbicides in Composting Feedstocks". The EPA has been initiating a registration review by doing a public docket. This docket allows for public comments and concerns to be taken into account. "Several composters and the US Composting Council have filed public comments on the dockets for all three persistent herbicides – clopyralid, picloram, and aminopyralid – requesting EPA withhold approval of reregistration of these persistent herbicides due to the potentially adverse effects on composting facilities and composts"(Coker, 2015). The use of carbon wood ash in composting is one possible method to reduce herbicide residues. The carbon wood ash acts as an activated carbon and bonds the herbicide to the carbon molecules (Coker, 2015). This method has been tested by Frederick Michel and his results show that "adding this type of ash at 2 percent by volume, the No-Observed Adverse Effect Level (NOAEL) rises from a concentration of 0.5 ppb to 10 ppb and with activated carbon, the NOAEL rises to 100 ppb" (Coker, 2015). The negative impacts of herbicides in compost affect every aspect of a company. GMC tests for herbicides using a bioassay method and Dan Goossen who is the General Manager of GMC said that adding ash to the compost mix adds $1.00 to $2.00 a ton to the processing costs. He discusses the loss of customers and how a lot of their customers came back because of being honest about the condition of their compost which seems like the best route to go when discussing herbicides in compost.

Another article goes in depth on a situation involving Aminopyralid. "A non-profit that operates several organic farms surrendered its organic certification in act of protest in July following severe crop damage linked to herbicide-tainted manure and compost used on those farms" (Sullivan, 2010). The response from Dow AgroSciences stated that "isolated crop damage that had been conclusively linked to the herbicide elsewhere in the U.S. and abroad was due to users not following proper "stewardship protocols" (Sullivan, 2010). This response to the concerns of the contamination of herbicides shows that there should be more readily available tests that allow users to test compost before it is applied to their crops.

Dr. Frederick C. Michel has published many articles and studies on this topic and one study he helped published was "Microbial Degradation and Humification of the Lawn Care Pesticide 2,4-Dichlorophenoxyacetic Acid during the Composting of Yard Trimmings. This specific study looks at the extent of degradation and the fate of 2,4-D during the composting of yard trimmings. They tested the compost over a 50 day period and after that period they measured the Organic Matter (OM), pH, compost stability, MPN, and the fate of ^{14}C-2,4-D during yard trimming composting . Their results showed that almost 50% of the ^{14}C-2,4-D carbon is mineralized, 23% is converted to humic acids, and 19.5% is unextractable after composting (Michel et al., 1995). From this research, there is some hope that herbicides are able to break down to a concentration or completely break down so they can no longer affect the plants. Another study on herbicides was done on green beans, tomato, and cucumber plants. Each plant was planted in five different kinds of compost: compost without herbicide, with herbicide

Clopyralid, Trifluralin, Imprelis, and mixed herbicides (Robert et al., 2017). Their results showed that the green bean plants showed little difference between the different treatments. “All of the rates were almost above 90% which suggested that when the compost addition ratio was no more than 20%, the herbicides in compost and the compost addition ratio both had no inhibited effect on its emergence rate, even the mixed herbicides in compost” (Robert et al., 2017). The tomatoes showed more of a difference between the different composts, but the cucumbers, based on their graphs, showed significant differences, especially in C2 and CM. Their conclusions on their results were that “It could be concluded from present study that compost addition could increase the emergence rate (except green bean) and plant growth indexes even the addition ratio increased to 20%, while the inhibitory of the residual herbicides in compost for tomato and cucumber were more obvious than that for green bean” (Robert et al., 2017). The results do show that some plants are more sensitive to herbicides than others, but the importance of testing and understanding the effects of herbicides are key to consumers and facilities that use and make compost.

Eric P. Burkhart and Nadine H. Davitt wrote a case study about the Herbicide Persistence in Finished Compost Products. The authors did a case study on the research done at Penn State on composting. The results from the bell pepper plots did not show symptoms until 2000 even though the plots were established in 1999. The results of the research showed the “longevity and possible mechanisms for clopyralid persistence (Burkhart & Davitt, 1999). The possibility of herbicide drift was taken out of the equation due to careful planning and protected environment. The authors concluded that clopyralid was found to be resistant to degradation for more than two years following applications to high tunnel cropping systems.

Materials and Methods

Figure 2: *(Picture on the left) A close up of the control mix, clean compost, spiked compost, Pea seeds, DNC Fertilizer*

Figure 3: *(picture on the right) The materials that were in the Bioassay kit that was provided by CREF*

The materials that were used in this study were: the Aerogarden LED Light (45 Watt), light timer, seeding tweezers, waterproof labels, clear plastic tray dome, plastic pots (36), nesting tray for pots, base tray, Wando garden pea seeds (250 grams), dry nutrient charge (120 grams crushed), control mix: Sphagnum Peat (80%), Perlite (20%), lime & wetting agent mix (25L), 2.5 L of 400 ppb spiked compost, 7.5 L clean compost, Compost from the Knox Farm • Compost from Professor Hope's garden in Knoxville, 2 different batches of compost from Better Earth, HANNA Combo pH/Conductivity/TDS Tester (High Range).

For this research, 4 different batches of compost were tested. Compost from Knox College, a batch from Knoxville, Illinois which was a home composting bin, and two different batches from Better Earth, which is an industrial compost facility just outside of Galesburg, Illinois. The bioassay kit was provided by the Compost Research and Education Foundation (CREF). The kit included the Aerogarden LED Light (45 Watt), light timer, seeding tweezers, waterproof labels, clear plastic tray dome, plastic pots (36), nesting tray for pots, base tray, Wando garden pea seeds (250 grams), dry nutrient charge (120 grams crushed), control mix: Sphagnum Peat (80%), Perlite (20%), lime & wetting agent mix (25L), and 2.5 L of 400 ppb spiked compost, 7.5 L clean compost. 2 liters of the control mix was mixed with 3 grams of the DNC fertilizer. After that the spiked compost was mixed thoroughly with the clean compost to make 10 liters of 100 ppb clopyralid spiked media. The HANNA Combo pH/Conductivity/TDS Tester (High Range) was used to take the electric conductivity of every batch of compost. This was done to test for the pH of each compost so that the correct amount of control mix could be mixed in with each batch of compost. The table below is how we determined the 'recipe' for each compost.

Compost EC using 1:5 method (mmhos/cm)	Category	Need for dilution	Compost volume: Control Mix volume
0.0 -1.24	Acceptable EC	No need for dilution	2L compost
1.25 - 2.49	Low EC	Dilute 50/50 with Control Mix	1L compost: 1L control mix
2.50 - 5.00	Moderate EC	Dilute 25% Compost with 75% Control Mix	0.5 L compost: 1.5L control mix
> 5.00	High EC	Dilute 15% Compost with 85% Control Mix	0.3 L compost: 1.7L control mix

Figure 4: *The Media Preparation & Mixing table. This was used to verify the dilution of the control mix and different compost batches*

After each compost batch was mixed with the correct amount of control mix and 2 grams of the DNC fertilizer, each compost was then put into the potting cups. We filled four potting cups for each compost including the clean control and the spiked control. We decided to double the Knox College compost and the Knoxville compost, so those two had eight cups each. The pots were labeled with the 'Sample ID' and the 'Replicate' number. We chose to bottom water all of the cups so as not to accidentally contaminate any of the cups with the spiked compost because of possible water splashing out if we were to use the normal watering technique. Two pea seeds were then placed in each cup and the cup was then placed in the base tray using a layout the CREF provided. The layout is based on a staircase pattern and accounts for the variation of the lighting unit. The grow light was set on a timer to be on for 12 hours and after germination, the plants were grown for 15 days. During that 15 day growing period, the plants were monitored and observed. After the 15 days, the plants were removed from the base tray and the leaf count, leaf cupping, severe leaf cupping, along with the germination percentage, and herbicide damage (quality score 0-5) were all recorded. The mean quality, the percent of damaged leaves, and the percent of severely damaged leaves were calculated.

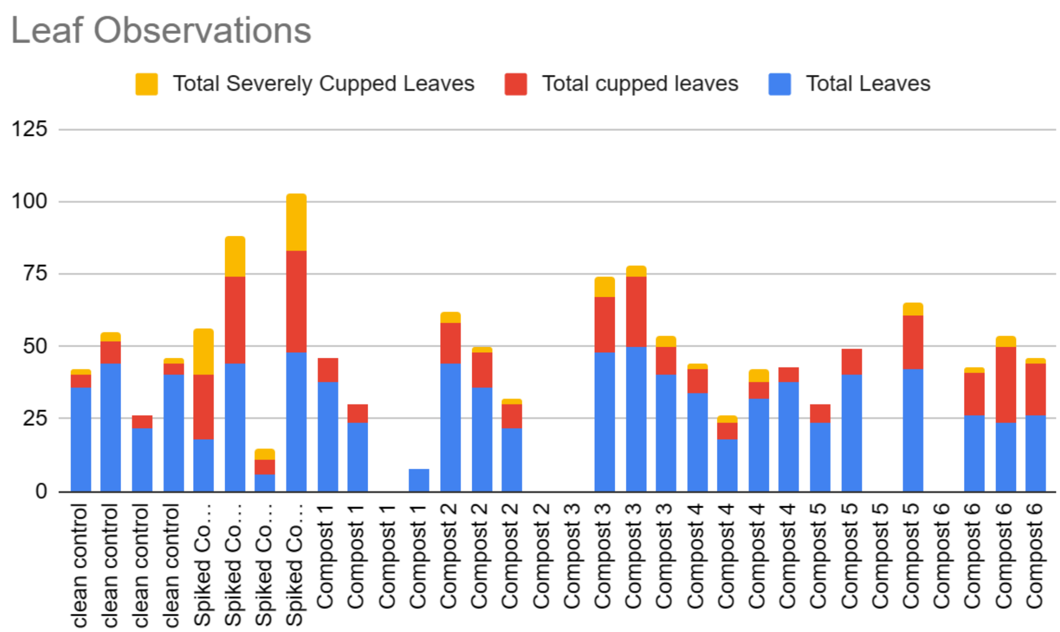
Rep 4 Sample ID 3	Rep 4 Sample ID 4	Rep 4 Sample ID 5	Rep 4 Sample ID 6	Rep 4 Sample ID 7	Rep 4 Sample ID 8	Rep 4 Sample ID 1	Rep 4 Sample ID 2
Rep 3 Sample ID 5	Rep 3 Sample ID 6	Rep 3 Sample ID 7	Rep 3 Sample ID 8	Rep 3 Sample ID 1	Rep 3 Sample ID 2	Rep 3 Sample ID 3	Rep 3 Sample ID 4
Rep 2 Sample ID 7	Rep 2 Sample ID 8	Rep 2 Sample ID 1	Rep 2 Sample ID 2	Rep 2 Sample ID 3	Rep 2 Sample ID 4	Rep 2 Sample ID 5	Rep 2 Sample ID 6
Rep 1 Sample ID 1	Rep 1 Sample ID 2	Rep 1 Sample ID 3	Rep 1 Sample ID 4	Rep 1 Sample ID 5	Rep 1 Sample ID 6	Rep 1 Sample ID 7	Rep 1 Sample ID 8

Figure 5: *Template for the placement of the soil cups in the tray*

Figure 6: *Final set up of the tray and grow light (March 29, 2023)*

Results

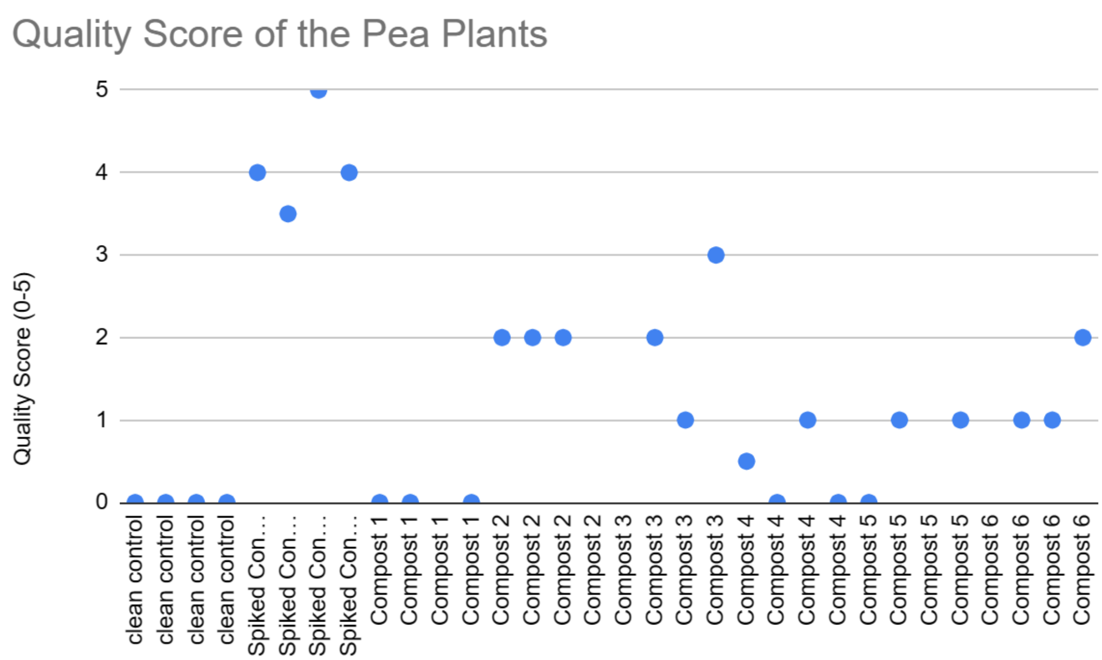
All of the plants had 100% germination except for Compost #1 which was the first set of Knoxville, Compost #2 which was the first batch from Better Earth, and Compost # 5 which was the second set of Knox College compost. For each of the compost batches, the separate plants did show signs of cupped leaves that resembled the indicator for the presence of herbicide, but the severity of those plants did not mirror the spiked control compost.



Graph 1: *Shows Leaf observations using a stacked bar graph*

The graph above shows the leaf observations. For the clean compost control, the total leaves for all four plants was 142. Of that 142, 20 of them showed cupping, and 7 of the total leaves were severely cupped. The spiked compost had a total of 116 leaves, the amount of cupped leaves was 92, and the amount of severely cupped leaves was 54 leaves. For compost #1 which was the first batch of Knoxville compost the total leaves came out to be 70, the number is so low due to the third replicate not growing at all. Out of the total leaves, 14 of them were cupped and none of them showed severe cupped leaves. Compost #2 was the first Better Earth batch and there were 102 total leaves, again there was one replicate that did not grow. The total cupped leaves was 30 and the total severely cupped leaves was 8. Compost #3 was the first batch of the Knox Farm composting and it had a total of 138 leaves. 53 leaves were cupped and 15 of the leaves were severely cupped. Compost 3 also had one replicate not growing at all which accounts for low leaf numbers. Compost #4 was the second Better Earth batch and it had 122 leaves in total, out of the total there were 25 leaves that showed leaf cupping and 8 that showed severe leaf cupping. Compost #5 was the second batch of Knoxville compost with a total of 106 leaves, 34 cupped leaves and 4 severely cupped leaves. The last compost, Compost #6 was the second batch of the Knox Farm compost. The total leaves for this compost was 76, the total cupped leaves was 59 and there were 8 severely cupped leaves.

After looking at the leaves it is important to note the gaps in the graph. The graph shows no data for replicate 3 for compost 1, replicate 4 for compost 2, replicate 1 for compost 3, replicate 3 for compost 5, and replicate 1 for compost 6. The reason that these data are missing is due to accidentally not planting the pea seeds in these replicates. Unfortunately, this does skew the results because if these were planted, there would have been more data to record and more observations to be made. Each plant was also observed for quality on a scale of 0-5. Zero being very healthy, no herbicides present and 5 being severely damaged by herbicides. For all four replicates for the clean control each scored a zero. For the spiked control all of the plants scored high with the highest being a 5 and the lowest a 3.5. Compost 1 scored all zeros, compost 2 scored 2 for each replicate, compost 3 scored a 2,1, and 3 for the respective replicates. Compost 4 replicates scored a 0.5, 0, 1, and 0, these plants all looked fairly healthy. Compost 5 scored well, two of the plants scored one and the other plant scored a zero and Compost 6 scored two 1's and a 2. The average of the separate compost batches quality scores was then calculated. The mean quality for the clean compost was 0, the spiked compost was 4.125, Compost #1 was 0, Compost #2 and #3 were 2, Compost #4 was 0.375, Compost #5 was 0.666, and Compost #6 was 1.333. Overall the compost batches scored well compared to the spiked compost, especially the first batch from Knoxville. The Graph Below shows the quality score of the pea plants just described above.



Graph 2: *shows the quality score of each plant*

Discussion

The Results from this study showed that each compost batch showed signs of possible herbicides, but unfortunately, there could be other factors that could account for some of the results found. The article "Herbicide Residues in Composts: pH and Salinity Affect the Growth of Bioassay Plants", written by W.F. Brinton, E. Evans, and T.C. Blewett, starts by stating one way other factors could affect plant growth. "The handling of composts in herbicide residue bioassays can be critical to the development of accurate results as composts often contain high salt levels at concentrations known to inhibit plant growth and development" (Brinton et al., 2005). Above are a few pictures of the plants after the 15 day period. The picture on the left shows compost from the Knoxville batch and the picture on the right shows plants from the Spiked compost. As you can see from the picture on the left, the plant looks very healthy, except for some of the leaves at the top of the plants. Those leaves are showing leaf cupping. The spiked compost shows severe leaf cupping. These two pictures are good representations of not only a healthy plant and an unhealthy plant, but also the effects of herbicides on plants.

Figure 7: *(April 13, 2023) Compost from the second batch of Knoxville Compost*

Figure 8: *(April 13, 2023) Spiked Compost control*

Future Directions

This bioassay kit could be used to test different plants and the use of a different herbicide. It would also be interesting to allow the plants to grow longer. The grow light would have to be either adjusted or removed after a certain amount of days because the Wando Pea plants that were grown ended up growing through the holes in the dome and getting too close to the grow light which damaged a lot of plants due to the heat it was giving off. Fine tuning the bioassay kit that was used would help hopefully simplify the process of detecting herbicides. One future idea would be to take the plants that were grown under the grow light and allow them to grow to full maturity and then observe the effects of the herbicides on the vegetables grown.

Conclusion

The main idea is that we can't state definitively whether herbicides were present or not without further tests, but we can point to the observations. We have to be open to the possibility that the cupped leaves also might indicate other factors affecting the plants. The results from this study showed that herbicides could be measured using a simple bioassay kit. It also showed that herbicides could be Potentially be present in small quantities. It is a good reminder of the importance of knowing the sources of compost feedstock and for the importance of regular testing.

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