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Final Report for;

Can physical contaminants (glass) in Mixed Waste Organic Outputs (MWOO) adversely effect the soil habitat?

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Executive Summary

Mixed waste organic outputs (MWOO) are the pasteurised and biologically stabilised organic outputs resulting from the mechanical biological treatment of mixed waste. Mixed waste refers to wastes containing putrescible organics that have been collected from households, litter bins, or certain commercial premises. These materials may also be mixed with manure, food wastes, animal wastes or source separated household garden and food waste.

Mixed Waste Organic Outputs have the potential to benefit agricultural production when used as soil amendments. However, they also contain physical contaminants such as glass, steel and hard plastics. In order to meet size restrictions, MWOO is often crushed during production. The resulting crushed or milled glass in the MWOO has been shown to be quite different in shape compared to the rounded edges of soil particles. This difference may mean that the addition of the glass in MWOO to soils, has the potential to harm human health through physical contact, and may also cause damage to the soil habitat for soil biota or growing crops, or may cause harm to grazing animals. One of the peak bodies representing livestock production in Australia, (Meat and Livestock Australia – MLA), now require livestock producers to provide grazing conditions that comply with physical contaminant restrictions in grazing paddocks, in order for the granting of livestock certification for stock from that grazing property.

Regulations governing MWOO application in NSW currently permit applications of up to 3.5 t/ha of glass to mine sites, as an incidental contaminant, 0.75 t/ha to plantation forestry and non-contact agriculture, and 0.15 t/ha for broad acre agricultural land, assuming a maximum total glass content in the MWOO (>2mm) of 2.5% for mine sites, and 1.5% for plantation and broad acre agriculture. However, the occurrence and incidental application of glass contaminant of a size < 2mm is not regulated and industry uses size reduction equipment (milling) to reduce amounts in the > 2mm fraction in order to meet application rate regulations (EPA personal communication).

We sought to separate glass effects from those of the MWOO as a whole, and so the experiments we carried out used increasing amounts of only crushed glass. In an attempt to replicate the glass size reduction process carried out by industry, we used a jaw crusher system to produce crushed glass which was initially > 2mm diameter and was further reduced in size so the majority was < 2mm. Therefore, this process resulted in the production of material of both > 2mm and < 2mm, with the proportion of glass in the two size fractions found to be in the approximate ratio 2:1 (< 2mm:> 2mm). Accordingly, this ratio was used to formulate the glass treatments added to the soils used for this project. All of the glass application rates used in our trials were based on the amount of glass in the > 2mm fraction.

The data presented in this report is for three experiments aimed at assessing the possibility that milled glass, similar to that found in MWOO, may adversely affect the soil habitat for; soil biota (worm avoidance), soil microbial populations (rhizobia nodulation) and agricultural crop production (carrot trial, tuber vegetables).

This study suggests that there is minimal effect of glass addition on earthworm behaviour, at MWOO application rates that are currently allowable. Some avoidance behaviour was observed for the 200 t glass / ha treatment, but this rate is currently higher than would be expected to occur in any MWOO application scenario. Similarly, we found no effect of glass application on legume nodulation, for MWOO applications allowed under current legislation.

Added glass was observed on the surface of carrot tubers harvested from glass amended soils. Glass was also isolated from soils adhered to tubers at harvest and in peel separated from these tubers. Glass was found on carrot tubers at the lowest glass application treatment used (0.25 t > 2mm glass / ha). However, this glass rate is equivalent to 25 t MWOO /ha; a rate of MWOO application that is higher than that allowed for broad acre agriculture under current regulations. It

should also be noted that, because the glass application rate of 0.25 t/ha was based on the amount of glass in the > 2mm fraction, the total glass applied in this treatment actually exceeded 0.6 t/ha, because it also included an amount of glass in the < 2mm fraction.

Given that this model study was conducted under controlled conditions using glass-only amended soils, it is recommended that the impact of glass on tuber vegetables such as carrots be verified under field conditions where actual MWOO has been applied. At the same time, it should also be noted that there has been no assessment of the impact of glass particles on livestock health and production in grazing systems.

Project activities undertaken to date

All project activities as set out in the project proposal have been completed. These include;

- preparation of milled glass
- worm avoidance experiment
- rhizobia nodulation experiments, including clover glass house trial, nodulation evaluation and counts of viable rhizobia
- Carrot glass house trial including evaluation of glass in soil adhered to carrots and detection of glass associated with carrot tubers

An evaluation of your compliance with the timetable set out in your application

All tasks have been completed

A description of any difficulties and/or delays you have encountered or expect to arise
See above

An outline of modifications/variations to your project that you have undertaken, or you intend to undertake, with the Trust's approval, to deal with difficulties or delays or which may improve the project's outcome

There were no planned modifications to the project

Contents

Research Report

Background

Mixed waste organic outputs (MWOO) are the pasteurised and biologically stabilised organic outputs resulting from the mechanical biological treatment of mixed waste. Mixed waste refers to wastes containing putrescible organics that have been collected from households, litter bins, or certain commercial premises. These materials may also be mixed with manure, food wastes, animal wastes or source separated household garden and food waste.

When applied at suitable rates, soil amendment products made from organic wastes, such as MWOO, have the potential to benefit both consumers and the community; not least of which is their benefit to agricultural production. However, there may be a downside to the use of these materials and this includes the addition of physical contaminants such as glass, which is often crushed during MWOO production, in order to meet size restrictions. The microscopic view of crushed or milled glass shows sharp and jagged morphology, and this is quite different from the typically rounded edges associated with weathered particles found in soil.

The 2014 NSW EPA Resource Recovery Order, pertaining to organic outputs derived from mixed waste (EPA 2014) currently permit applications of up to 3.5 t/ha of glass contaminant to mine sites, 0.75 t/ha to plantation forestry and non-contact agriculture and 0.15 t/ha for broad acre agricultural land. All of these land use scenarios can potentially include management systems containing pasture-based agriculture.

While there has been a concerted effort to quantify risks from chemical contaminants in soils, less critical analysis has been given to the addition of physical contaminants such as glass. The addition of physical contaminants to soils has the potential to harm human health through physical contact and may also cause damage to the soil habitat for growing crops (Terman and Mays, 1973), or soil biota (Stamatiadis and Dindal 1990), and may also cause harm to grazing animals (Krause et.al. 1996; Chanie and Tesfaye, 2012). Once this glass is added to the soil it cannot be removed without removing the soil itself

Hoet et.al. (2004) reviewed the potential human health risks from nanoparticles (very fine; 10^{-9} m = 1 nm) and concluded that particles in the nano-size range may enter the body via the lungs or intestinal tract, and that distribution within the body depend on their size. Anderson and Karmali (2013) reviewed the medical literature on the occurrence and treatment of patients following glass ingestion by adults. They concluded that while in the majority of times (80-90%), ingested objects pass through the digestive tract, 10-20% of patients required some sort of endoscopic retrieval.

Recently, Abrahams (2013) reviewed the risks from the involuntary ingestion of soil by livestock and humans, and indicated that materials present in ingested soil would end up in the gastrointestinal tract. The World Organisation for Animal Health includes the presence of physical contaminants in stock feed, in its Terrestrial Animal Health Code, stating that measures must be made to prevent physical hazards that may occur in feed and feed ingredients (OIE 2011). It is also well known that grazing livestock ingest considerable amounts of soil during their foraging activities. It has been shown that a sheep diet can contain up to 14% soil on a dry matter basis, while for grazing cattle; this figure can be up to 8% soil, on a dry matter basis (Davis et.al. 1986). One of the peak bodies representing livestock production in Australia, (Meat and Livestock Australia – MLA), now require growers to ensure that their livestock have not been exposed to potentially injurious physical contaminants. This requirement forms a part of MLA's Livestock Production Assurance

Program (LPA) and every accredited producer must undertake to minimise the risk of livestock being exposed to sites that are unacceptably contaminated with persistent chemicals or physical contaminants (see - http://www.mla.com.au/globalassets/mla-corporate/generic/meet-safety-and-tracability/lpa-factsheet-propertyriskassessment.pdf.).

Vegetable crop production may also be at risk if glass shards become adhered to, or embedded within, root and tuber crops. This has the potential to permanently prevent the production of these crops for human or animal consumption, in soils containing physical contaminants. For example, food processors and manufacturers are becoming increasingly conscious of the public perception and potential health risks surrounding a variety of contaminants in food, and vendors supplying food to these organisations must adhere to strict quality assurance conditions (e.g. Woolworths 2013; Quality Assurance manuals for Primary Production Produce; Quality Assurance manuals for Manufactured Foods). Both of these Quality Assurance Standards state that "all products shall be free of extrinsic foreign objects such as plastic, glass, metal, dirt, or grease, including contamination from the process or packaging".

Pasture and cropping systems that rely in part on the fixation of atmospheric nitrogen by nodulated legumes may also be adversely affected by physical contaminants. Legume crops play a vital role in Australian agriculture because of their ability to fix atmospheric nitrogen into nodules on their root systems that the plants can then use. Crop rotations including leguminous species therefore play a significant part in restoring nitrogen levels in soil. The success of the nodulation process is particularly moisture dependant (Slattery et.al. 2001), and so this process may also be at risk from physical contaminants, as it is possible that these particles my compromise the nodule structure, thus leading to desiccation. It is unclear what effect physical injury during nodule inoculation and growth will have on rhizobia effectiveness or survival, and to the best of our knowledge, this has not been investigated previously.

The objective of this project is to carry out an initial study using a model system to determine the likelihood that glass contaminants in MWOO adversely affect soil health, by examining the effects of added glass on;

- legume-rhizobia nodulation;
- plant roots / plant tubers; and
- soil biota such as earthworms

Materials and Methods

This project is divided into three rate response experiments, each of which was carried out under controlled conditions. These experiments are listed below;

1. Worm avoidance test as an evaluation of glass effects on soil biota;
2. Rhizobial effectiviness as an evaluation of glass effects on rhizobia nodulation and their ability to fix atmospheric N; and
3. Carrot pot trial as an evaluation of the potential impact of glass contaminants on the quality of tuber crops.

With the aim of separating any glass effects from those of the MWOO as a whole, these experiments relied on the addition of increasing amounts of milled (crushed) glass only. The milled glass was prepared by crushing clean glass to a size range that falls within limits specified under the 2014 NSW EPA Resource Recovery Order, pertaining organic outputs derived from mixed waste (EPA 2014); where application limits were based on the amount of glass in the > 2mm fraction. Once prepared, the glass was added to potted test soils at rates up to and above those rates allowable under current regulations governing the application of MWOO to soil, where 3.5 t/ha of glass contaminant can be applied to mine sites 0.75 t/ha to plantation forestry and non-contact agriculture and 0.15 t/ha for broad acre agricultural land. All expermental designs are fully replicated using statistically validated methodology and designs.

Preparation of milled glass

The range of glass application rates (0 - 20 t glass/ha), were based the amount of glass in the > 2mm fraction, which is also the basis for NSW regulations governing glass inputs to soil. To prepare the milled glass, clean boro-silicate glass jars were broken up using a mallet and then the pieces passed through a Retsch BB51 Jaw Crusher with jaws set to a minimum closure width of 2mm. The milled material was sieved to 2mm and the remaining > 2mm fraction was again passed through the jaw crusher and again sieved to 2mm. This yielded amounts of milled glass in the > 2mm and < 2mm fractions approximately in the ratio 2:1, (see Table 1), and therefore the glass which was added to the test soils, also included an amount from both the < 2mm fraction (62%) and > 2mm fraction (38%).

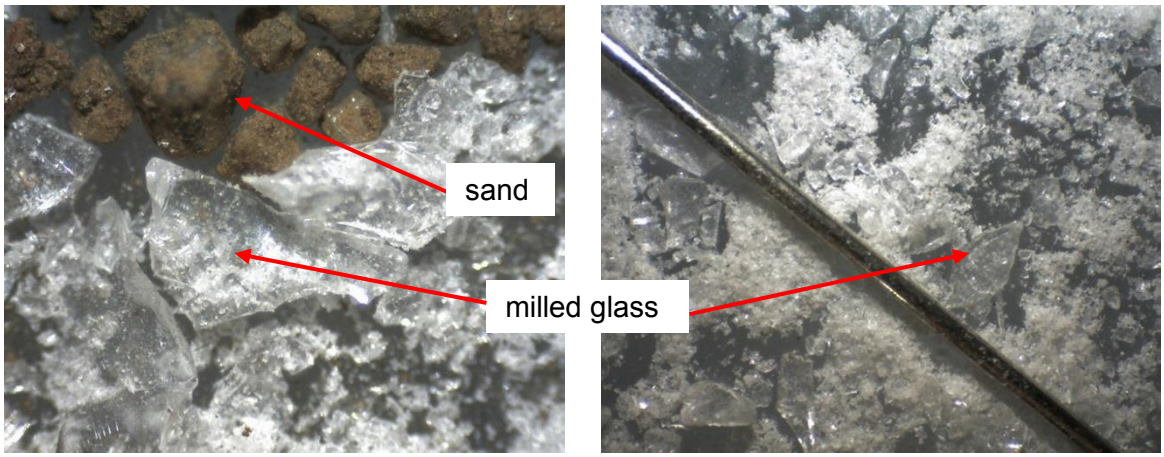
Table 1: Size fractionation (>2mm and < 2mm) of milled glass used in these experiments

Size fraction	%
Glass mixture	
>2mm	33 – 38
<2mm	62 - 67
<2mm fraction	
2 – 1 mm	23
1 – 0.5 mm	18
<0.5 mm	26

A further size fractionation was carried out on subsamples of the milled glass mixture that was prepared above. Using a nest of stainless steel test sieves, the proportion of glass present in size fractions > 2mm, 2 – 1mm, 1- 0.5 mm and < 0.5 mm was measured. These data are also presented in Table 1. The fractions visible to the naked eye (> 0.5 mm)

comprise nearly three quarters (74%) of the glass added to the test soils, with around one quarter (26%) being visible via a microscope.

The microscopic view of the milled glass (Plates 1a and 1b) shows sharp and jagged morphology, and this is quite different from the typically rounded edges associated with weathered sand particles found in soil (Plate 1a). This morphology is similar to glass fragments previously isolated from the MWOO material (Cattle, 2016).



Plates 1a and 1b: Comparison of milled glass with sand (Plate 1a). Particle size distribution found in a subsample of milled glass. Household steel pin used as a size comparison (Plate 1b).

Preparation of soil treatments

The soil used in the three experiments is an Alfisol, or a Red Chromosol (Isbell 1996). It was collected from the Night Paddock at the Centre for Recycled Organic in Agriculture (CROA) site, Menangle, near Camden NSW (70m AHD; 02883278E, 6224546N). This soil had previously been shown to present a favourable habitat for plant growth, soil fauna (worms) and soil microbial populations (Whatmuff et.al. 2005; Warne et.al. 2008; Heemsbergen et.al. 2010). Basic chemical, physical and morphological properties of this soil are described in Appendix 1. The soil is moderately acid throughout (pH measured in 0.01*M* $CaCl_2$, [pH_C], 5.6 in the A horizon to pH_C 6.4 in the subsoil) and has moderate levels of fertility and organic carbon levels (OC 2.1 %). The soil also has low background concentrations of Cd (0.05 – 0.09 mg/kg), Cu (17 - 21 mg/kg) and Zn (40 – 65 mg/kg) (see Appendix 1).

Soils for these experiments were prepared from a bulked composite sample (100 kg) collected from the top 10 cm. The soil was dried under forced draft (40°C) and ground to pass through a 2 mm sieve, followed by thorough mixing to ensure homogeneity. Once homogenised, appropriate amounts of milled glass was added to approximately 10 kg of soil, depending on the specific treatment (see Tables 2, 3 and 4), and the amended soils again thoroughly mixed. Soils were stored in airtight containers until required.

In preparing the treatments, we assumed that 1 ha of land is equivalent to 1000 t of soil (7.5 cm thick with a bulk density of 1.33 g/cm^2).

Worm avoidance

We used a standardized earthworm avoidance test (ISO 17512-1 2008) to provide information on whether the milled glass applications had an effect on the habitat of soil

animals. This test allows for a rapid determination of changes to the soil habitat and a high degree of sensitivity to applied treatments (Hund-Rinke and Wiechering 2001). The basic premise behind the avoidance test, is that worms will avoid unfavourable habitats (or less favourable conditions), when given a choice. The test species used was Eisenia fetida (*E. fetida*), also known as 'tiger' or compost worms. The only difference between the test protocol and the method we used is that, rather than using an artificial soil as specified, we opted for the field soils collected from the experimental site as described above.

The avoidance tests were carried out after the completion of suitable range finding tests as recommended in the ISO standard. These are used to determine if the worm will actually survive the experimental treatments. Mortality of more than 10% of the worms in the avoidance test invalidates the test for that treatment. An acute mortality test carried out on pure milled glass, showed 100% worm survival when worms were exposed to milled glass only for up to 72 hours. In addition, all test batches included a reference toxicant treatment (boric acid H_3BO_3), applied at a rate of 750 mg H_3BO_3 /kg soil. Boric acid has been used historically as a soil chemical sterilant and is an effective non-selective biocide. More than 80% of worms should avoid the reference toxicant (boric acid) treatment.

Treatments used for the worm avoidance experiments are sumarised in Table 2 below. While similar glass application rates were used for all three experiments the worm avoidance trial had an additional rate of 200 t milled glass / ha.

Table 2: Summary of treatments used in the worm avoidance experiments

TRT #	Rate >2mm glass t/ha	Worm avoidance reps
1	0	5
2	0.25	5
3	0.5	5
4	1	5
5	3	5
6	5	5
7	10	5
8	15	5
9	20	5
10	boric acid	5
11	200	5

Each test was replicated 5 times and spaces for each container were randomly allocated using a completely randomised block design. Subsequently, soils were equilibrated with the applied treatments for 1 week prior to adding the worms. For this step, soil moisture was maintained at 90% maximum water holding capacity. The test boxes consisted of two chambers (Plate 2a), with the test material and the control soil separated by a divider, prior to addition of the worms. Once the divider was removed, ten mature worms were placed on the soil surface in the centre of each container (Plate 2b) which was lidded once the worms were observed to enter the soil (Plate 2c). The tests were housed in constant temperature room (20 ± 2 °C) which was uniformly lighted (800 lx) at a controlled light/dark cycle of 16 h light to 8 h dark (Plate 2d).

The controlled lighting ensured that worms remained in the test medium throughout the test. After 48 h exposure to the test treatments, the barrier was reinserted into the container midline, and worms were counted in both the control and test soils, and percentage avoidance calculated (Plate 2e). Any occurrence of midline worms were split 50:50 between the control and test soil.

During the worm avoidance tests, all of the validity criteria for the worm avoidance test, as specified in the standard ISO method were met namely;

- mortality in any of treatments was always no more that 10%;
- worms avoided the reference toxicant, with more than 80% preferring the control soil; and
- worms were evenly distributed when the control soil was paced in both sides of the test boxes (± 20% avoidance/preference).

Plate 2 (a) Two sided test box showing divider separating control and test soil (L), (b) worms placed on mid line and (c), perforated lid replaced. (d) test boxes arranged in cool room and (e) after 48 h soils separated and worms on each side counted.

Plate 2(a)

Plate 2(b)

Plate 2 (c)

Plate 2(d)

Plate 2 (e)

Rhizobia nodulation

Treatments used for the glasshouse rhizobia innoculation experiments are sumarised in Table 3 below. This experiment used 10 treatments in total, including an inoculated and an un-inoculated control, both with zero glass. Each of the treatments was replicated three times with three pots per replicate, totalling 90 pots (Table 3). Pots were 15 cm in diameter. The soil was added to these in a manner that resulted in a consistent bulk density for each (~1.2 g/cm^3), by adding the same weight of soil to each pot and carefully packing to a standard height. The pots were laid out on glasshouse benches in a completely randomised block design. The glasshouse temperature was maintained at 25 ± 5°C. Soil moisture was maintained at 60% field capacity by watering to weight.

Table 3: Summary of treatments used in the rhizobia inoculation experiments

TRT #	Rate >2mm glass	Rhizobia trial		
	t/ha	inoculated	reps	pots/rep
1	0	Y	3	3
2	0.25	Y	3	3
3	0.5	Y	3	3
4	1	Y	3	3
5	3	Y	3	3
6	5	Y	3	3
7	10	Y	3	3
8	15	Y	3	3
9	20	Y	3	3
10	0	N	3	3

Inoculation of potted soil

To ensure adequate nodulation of seedlings, freeze-dried inoculant of subterranean clover, strain WSM1325 (BASF) was suspended in sterile deionized water and mixed to form an inoculant broth (Vincent 1970). All replicated pots of soil except the un-inoculated controls (treatment 10) were inoculated with approximately 310 mL broth 24h prior to sowing. Treatment 10 was supplied with 310 mL sterile water. The number of rhizobia in the broth was counted by distributing serially-diluted aliquots of broth onto the surface of specialized agar medium in Petri plates. The plates were incubated for 3 days at 26ºC and the number of rhizobia colonies counted. The number of rhizobia was > 3 x 10^9 per mL which supplied a calculated 4.28 x 10^5 rhizobia per gram soil in each pot.

Sowing of seed

Seed of subterranean clover (*Trifolium subterraneum*) cv. Goulburn was surface-sterilized and pre-germinated for 2 days before sowing 6 seedlings aseptically into each pot. The un-inoculated seedlings were sown first. After seedling establishment, the number of plants per pot was reduced to three. All pots were subsequently watered to field capacity soil moisture content every 2-5 days throughout the experiment.

Nodulation and plant biomass

Plants were harvested after 14 weeks growth. Standard protocols were used to score root nodulation on a scale of 1 to 5 (5 the best) and the root nodule location noted as either

crown or lateral (Yates et.al. 2016). Shoot dry weights were recorded after plant tops were cut and placed in a drying oven at 80-85ºC for 2 days before weighing.

Enumeration of rhizobia numbers in soils

The number of clover rhizobia present in the soil samples was determined using a most probable number (MPN) technique (Rockwell 1963). Briefly, 10g of soil was added to 90mL sterile deionised water and blended (stomached) for 60s to form a suspension. The suspension was diluted in 6 x 10-fold dilution steps. Aliquots from each dilution step were used to inoculate three replicate seedlings of subterranean clover growing aseptically in nitrogen-free plant agar in test tubes. The inoculated seedlings were placed in a temperate controlled environment room for 42 days after which time the nodulation was scored. The distribution of positive (nodulated) test plants is used to estimate the most probable number of clover rhizobia per gram soil.

Carrot trial

Treatments used for the carrot glasshouse trial are sumarised in Table 4 below. This experiment used 9 treatments with four replicates. Pots used were 15 cm in diameter and were laid out on glasshouse benches in a completely randomised block design. The soil was added to these in a manner that resulted in a consistent bulk density for each (~1.2 g/cm^3), by adding the same weight of soil to each pot and carefully packing to a standard height. The glasshouse temperature was maintained at 25 ± 5°C. Soil moisture was maintained at 70% field capacity (0.2 g/g), by watering to weight.

Table 4: Summary of treatments used in the carrot glasshouse trial

TRT #	Rate >2mm glass	Carrot trial
	t/ha	reps
1	0	4
2	0.25	4
3	0.5	4
4	1	4
5	3	4
6	5	4
7	10	4
8	15	4
9	20	4

Each pot was used to grow 2 carrots (*Daucus carota*) after initial thinning of emerged seedlings. We did not want plant nutrients to be limiting, so each pot received an application of nitrogen (N), phosphorus (P) and potassium (K) via a commercially available water soluble fertiliser NPK ratio 25:5:5.8. This was applied at 3 weekly intervals supplying 0.12 mg N per pot, 0.02 mg P per pot and 0.07 mg K per pot.

Carrot tubers were harvested manually and excess soil removed by tapping tubers onto a hard surface. Each of the tubers was then visually assessed for the presence of visible glass on the tuber surface or in the soil still adhered to the tuber surface. This remaining soil was washed from the carrot tubers and collected for analysis. The washed tubers were then peeled, and the peel and tuber material weighed and then dried under forced draft to constant weight (70°C). The plant material could not be mechanically ground as this would potentially damage any glass particles that were present. Therefore, all plant samples (peel and tuber) were combusted in a muffle furnace (500ºC for 4 hours), in order to reduce the

organic content while leaving any glass particles intact. The presence of any glass in these samples was qualitatively assessed using microscopy.

The soil adhered to the carrot tubers was dried under forced draft (40°C) and weighed. As with the plant material, these samples could not be mechanically ground as this would potentially damage any glass particles that were present. Instead, the soil samples were microwave-digested in reverse *aqua regia*, in order to remove oxides and other coatings which may mask the presence of any glass particles present. Following this digestion process, the 'clean' soils were subjected to the same size fractionation process used in preparing the milled glass using a nest of stainless steel test sieves and the proportion of glass in size fractions > 2mm, 2 – 1mm, 1- 0.5 mm and < 0.5 mm was measured by weighing the amounts of glass found in each fraction as isolated by the test sieves.

Statistical analysis

The statistical designs used throughout the experiments enable evaluation of data by analysis of variance (ANOVA), to show the response to the applied treatments. Least significant difference test (l.s.d.) was used to compare differences in treatment means at the 5% ($P<0.05$) level of significance and therefore the level of significance is indicated by the l.s.d. or standard error bar (s.e.).

Where appropriate, data was analysed using linear regression of each individual sample point, with regression coefficient (R^2) included as an indication of goodness of fit at $p<0.05$.

Results and Discussion

Worm Avoidance

A summary of the worm avoidance tests for all of the milled glass treatments, including an additional very high rate (200 t glass /ha) are given in Table 5 and are also illustrated in Figure 1.

Table 5: Treatment averages for worm avoidance tests carried out on milled glass amended soils.

Treatment #	Glass rate[1]	Avoidance	s.e.d.
	t/ha	%	
# 1	0	-4%	0.17
# 2	0.25	-16%	0.16
# 3	0.5	-12%	0.14
# 4	1	-6%	0.23
# 5	3	0%	0.19
# 6	5	-12%	0.15
# 7	10	-4%	0.17
# 8	15	4%	0.16
# 9	20	-12%	0.12
#10	200	35%	0.15
	Boric Acid	88%	0.05
	l.***s.d.***	**42.1%**	

[1]t/ha = based on application rate of >2mm glass and also contains glass < 2mm. l.s.d.. = standard error and indicates statistical significance at p>0.05

Included is the standard error of the difference between means (s.e.d.) for each treatment mean, and the l.s.d. which indicates significance at p<0.05. Treatments with an avoidance % in the positive range indicate avoidance of treatment, while treatments with an avoidance in the negative range, indicates preference for test treatments. Responses in the range ± 20% represent no avoidance or preference.

There was no avoidance behaviour observed for any of the milled glass treatments at application rates up to 20 t glass / ha, and no treatments resulted in the death of more than 10% of test worms. Some avoidance behaviour was observed for the 200 t glass / ha treatment, but this rate is much higher than would be expected to occur in any MWOO application scenario.

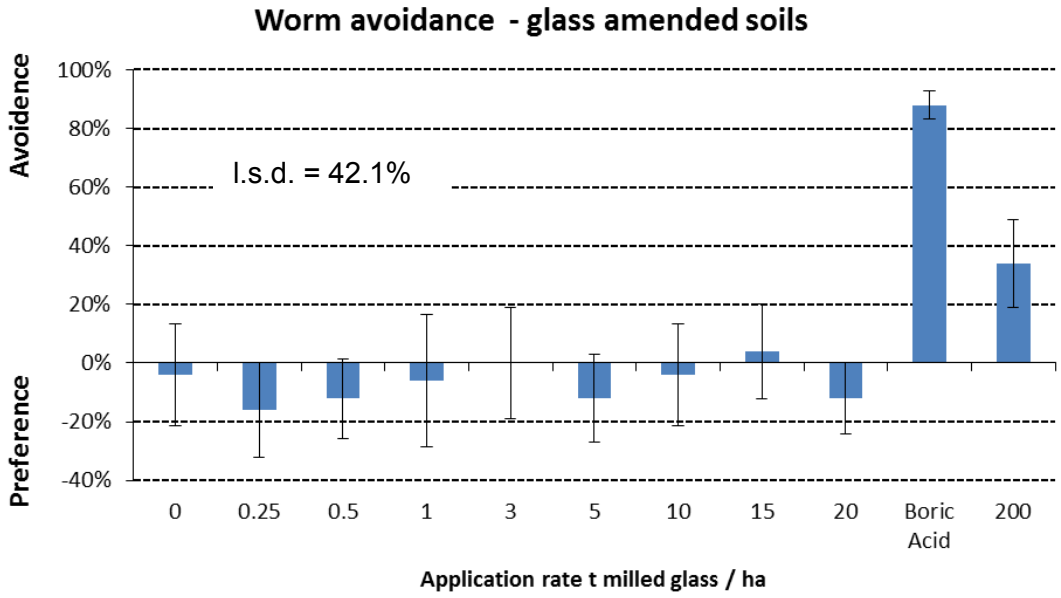


Figure 1. The effect of increasing rates of milled glass on worm avoidance behaviour compared to the control test soil. Positive range indicated avoidance of treatment, while negative range indicates preference for test treatments. Responses in the range ± 20% represent no avoidance or preference. L.s.d. indicates significance at p<0.05.

Rhizobia nodulation

Clover yield

The average effect of increasing rates of milled glass on clover yield is presented in Figure 2 and also Appendix 2. Analyses of the results for the analysis of variance of these data showed that there was no statistically significant effect of glass addition on clover yield, for the application rates used.

Nodulation

The average effect of increasing rates of milled glass on clover nodulation is presented in Figure 3 and also Appendix 2. As with the clover yield results presented above, results for the analysis of variance of this data showed that there was no statistically significant effect of glass addition on clover nodulation, for the application rates used.

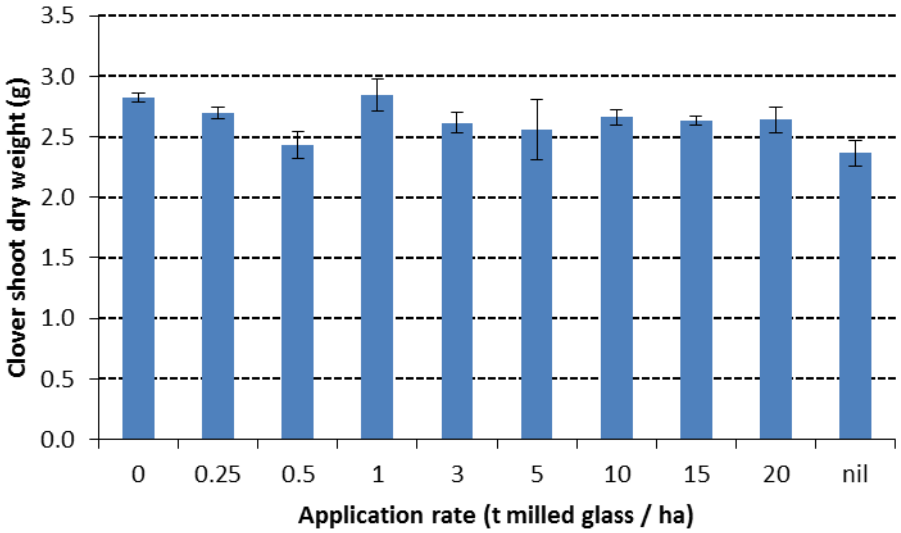


Figure 2: Effect of increasing application of milled glass on clover yield. Nil = un-inoculated control (zero glass).

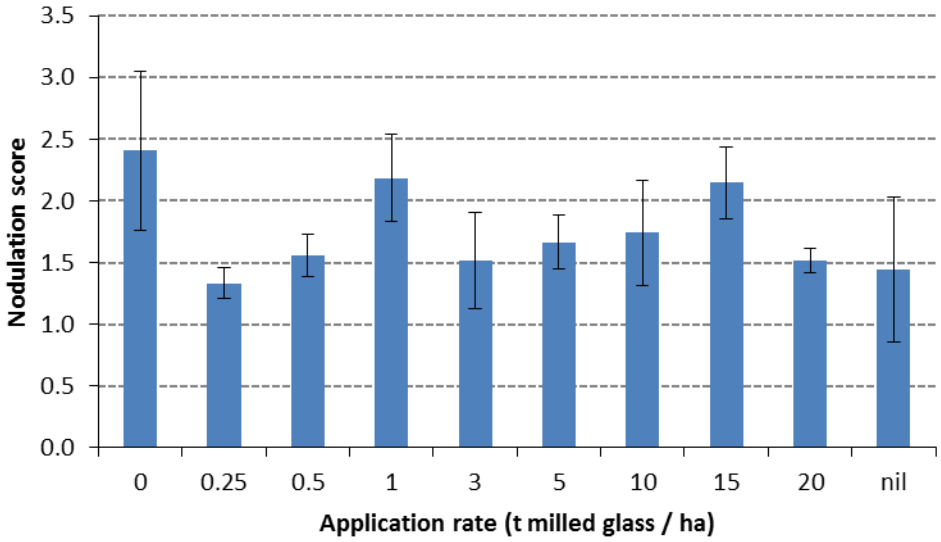


Figure 3. Effect of increasing application of milled glass on clover nodulation. Nil = un-inoculated control (zero glass).

Rhizobia counts (most probable number - MPN)

Generally, the rhizobia counts for all soils were low and were not affected by glass treatments (data not shown). There was no statistically significant effect of glass addition on rhizobia effectiveness.

Carrot glasshouse trial

Yield and soil adhered to harvested carrots

Average carrot yield, and the amount of soil that had adhered to the carrot tubers grown in the carrot glasshouse trial, is listed in Table 4. Also included in this table is an indication of

statistical difference between treatments (l.s.d.) at p<0.05. The amount of soil adhered to the harvested carrots, following initial removal of excess material, ranged from between 4.4g and 16.1 g per plant. This amount of soil that had adhered to carrot tubers was not influenced by glass application.

Table 4. Average carrot yield and amount of soil adhered to plants grown in the carrot glasshouse trial. Soils were amended with milled glass corresponding to rates of 0 – 20 t >2mm glass / ha. L.s.d indicates statistical significance at p<0.05.

Trt #	Rate (t glass / ha)	Dry soil on tuber (g)	Whole tuber (g)	Wet peel (g)	Dry peel (g)	Wet tuber (g)	Dry tuber (g)
1	0	15.4	60.5	10.5	1.3	46.8	6.2
2	0.25	16.1	44.6	12.8	1.3	36.1	4.9
3	0.5	4.4	42.3	10.0	1.1	28.6	4.5
4	1	10.5	74.4	15.7	1.4	59.0	6.8
5	3	11.8	47.3	10.9	1.1	36.7	4.9
6	5	7.3	53.9	12.6	1.4	41.4	6.0
7	10	15.5	59.4	12.9	1.2	45.1	5.6
8	15	7.3	57.4	11.8	1.1	35.8	4.2
9	20	13.8	62.7	12.6	1.2	49.3	6.1
	l.s.d.	***11.6***	***29.1***	***6.1***	***0.4***	***27.5***	***2.8***

Whole tubers varied in weight between 42.3 g and 74.4 g per tuber. Glass application had no effect on tuber weight.

Glass particles on carrot tubers and in associated adhered soil

Visible glass particles were observed on the surface of the carrot tubers and in soil adhered to tubers harvested from glass amended soil (Plates 3a and 3b) The occurrence of visible glass was observed at all rates of glass application.

Plate 3a. Glass on carrot tuber surface in plants harvested from glass amended soil

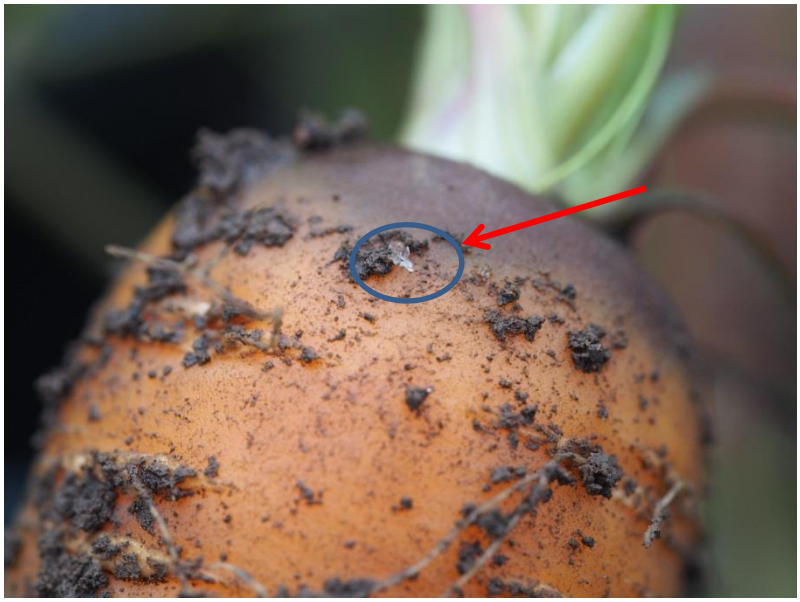


Plate 3b. Visible glass in soil adhered to carrots harvested from glass amended soil

Sieving of the soil adhered to the carrot tubers yielded amounts of glass ranging between <0.1 g to 0.9 g of glass per carrot. The amounts of glass measured increased with increasing rates of glass application (see Figure 4). However, our procedure (sieving), was not adequate to allow us to quantify the amounts of any glass particles present that were <0.5 mm. Particles of this size were observed using microscopy. As with the visual assessment of the harvested tubers, we detected glass particles in soils adhered to tubers from all glass application rates. The amounts measured agreed well with amounts predicted

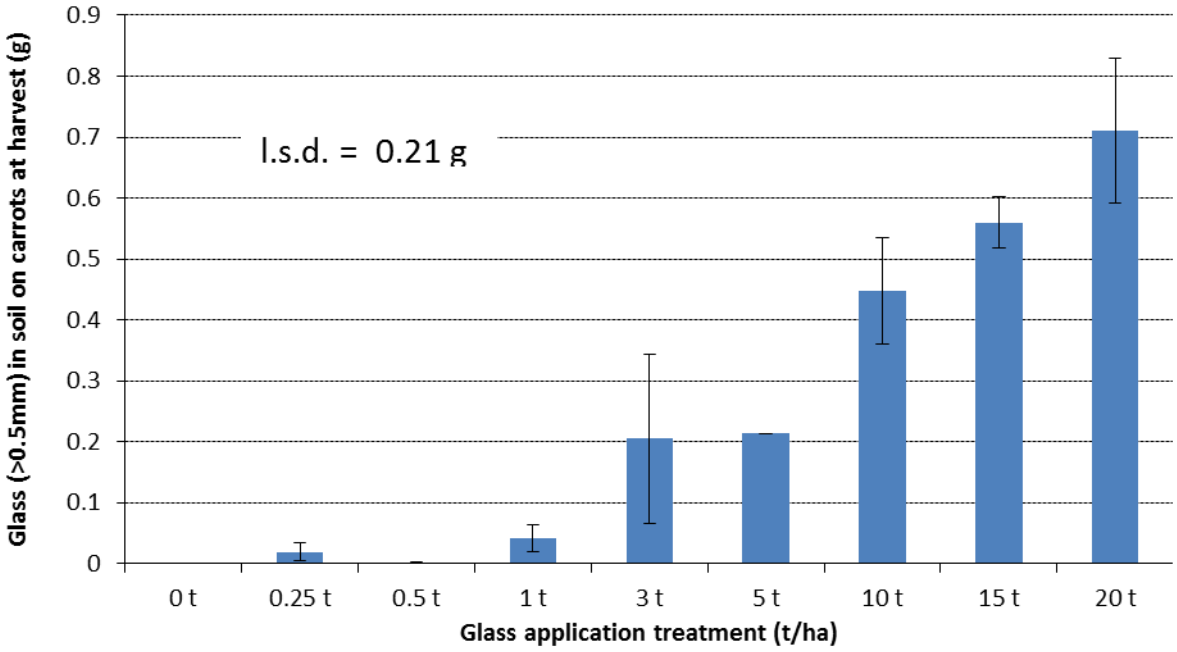


Figure 4. Average amounts of glass (>0.5 mm) in soil adhered to carrots grown in glass amended soils with increasing rates of application. L.s.d. represents statistical differences between treatments at p<0.05.

from the initial sieve analyses of the milled glass material (Table1). This result is quite encouraging, given how difficult it is to quantify the amounts of glass in various materials including MWOO (Echavarri-Bravo et.al. 2017), and verifies the procedures we used to physically measure glass found in soils and plant tissue for the carrot trial. The relationship between the amount of glass predicted to be in soil (>0.5 mm fraction), with that measured in the soil adhered to the tubers is presented in Figure 5.

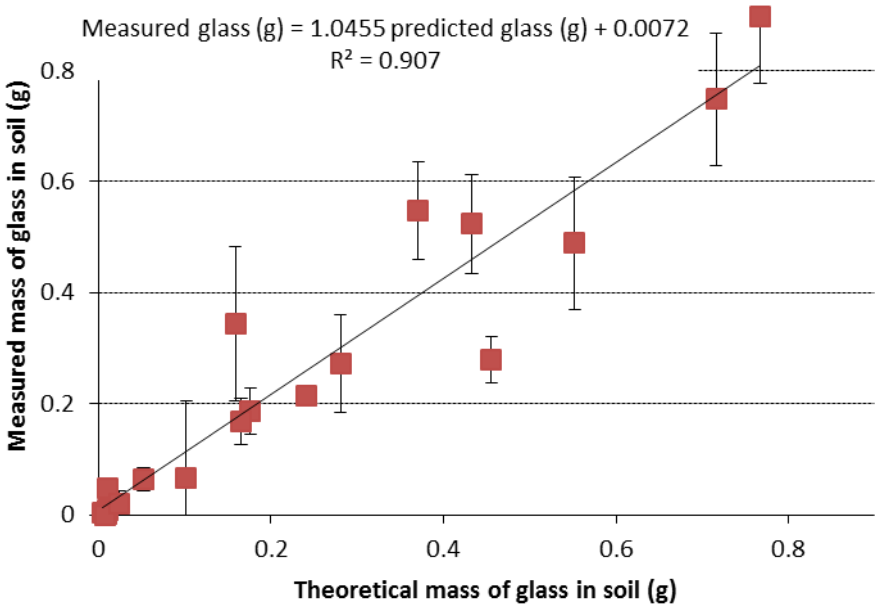


Figure 5. Quantity of glass (>0.5 mm) measured in soil adhered to carrots grown in glass amended soils, compared to amounts predicted from application rates and assessment of glass fractionation. Solid line represents statistical relationship between measured and predicted glass, with regression coefficient (R^2) included as an indication of goodness of fit at $p<0.05$.

Glass particles in carrot tissue

Fine glass particles (<0.5 mm) were observed in ashed carrot peel samples taken from plants grown in soils amended with the milled glass. Plate 4 shows the microscopic view of ashed carrot peel taken from zero glass treatment (control).

Plate 4. Microscopic view of ashed carrot peel taken from zero (control) glass treatment

This view can be compared with Plates 5a and 5b, which show glass fragments found in carrot peel taken from plants grown in soils amended with glass at rates of 5 t/ha (Plate 5a) and 20 t /ha (Plate 5b). However, we were not able to quantify these amounts of glass associated with the peel. It is also not clear whether the glass fragments observed were originally on the tuber surface and not removed with soil washing, or whether they had penetrated the peel itself. No glass was seen in the ashed tuber samples.

Plate 5a. Microscopic view of glass observed in sample of ashed carrot peel taken from 5 t/ha glass treatment

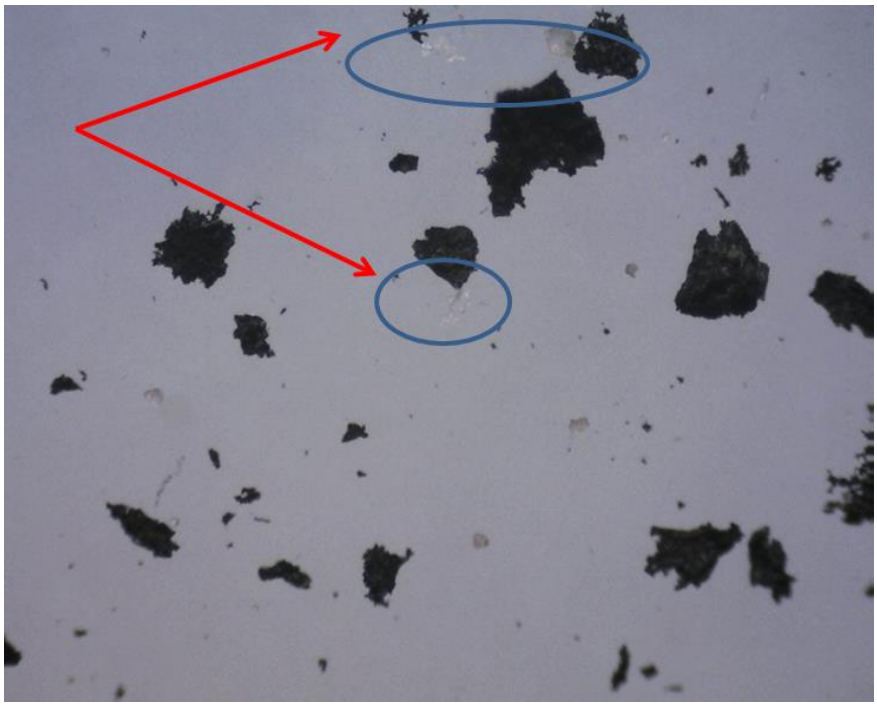
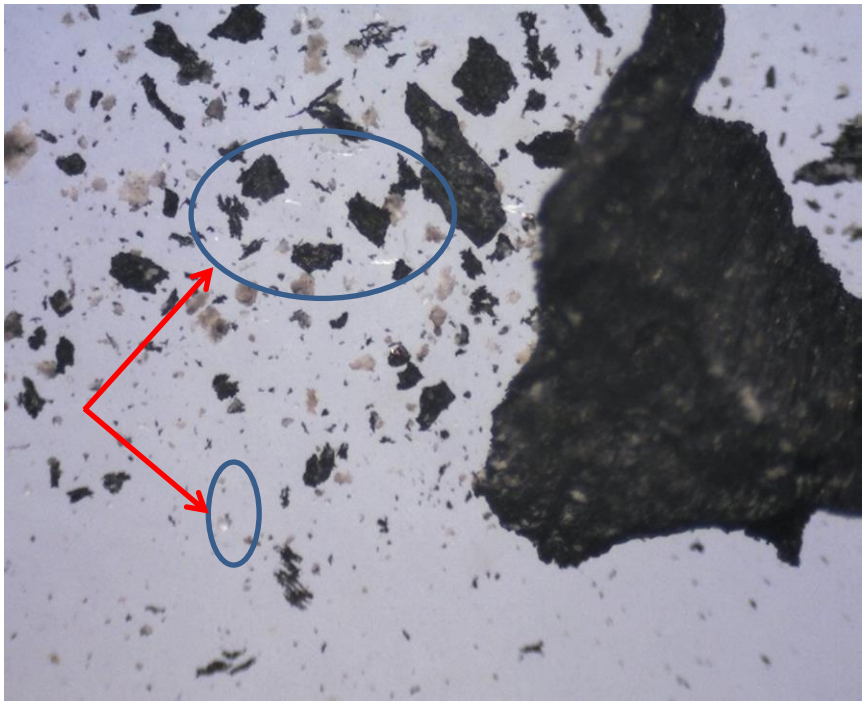


Plate 5b. Microscopic view of glass observed in sample of ashed carrot peel taken from 20 t/ha glass treatment



Converting glass application rates to rates of MWOO application

One of the major considerations of this project is to separate any glass effects from those of the MWOO as a whole; where it could be possible that any chemical contaminants in the MWOO may exacerbate any physical damage effects. Keeping this in mind, the experiments relied on the addition of increasing amounts of milled glass only.

The carrot, worm avoidance and rhizobia nodulation trials were designed to see if it was possible for glass added to soil to adversely affect the soil habitat. As such, some of the rates used were higher than are currently allowed under an MWOO land application scenario. To put the glass application rates into an MWOO land-application context, we converted the rates of applied glass into equivalent rates of MWOO application (see Table 5). In doing so, we made several assumptions, namely;

- The solid physical contaminants in MWOO (i.e. glass, metal and hard plastics), have a total solids content in the > 2 mm fraction of up to 2% as per analyses of material received;
- One half of this solid content is glass;
- Milling glass to reduce the amount of material in the > 2mm fraction results in significant amounts < 2mm, and the proportion of this < 2mm fraction is similar to that seen when we prepared milled glass treatments (62 % < 2mm : 38% > 2mm); and finally,
- There is a proportion of fine glass (as much as 26% < 0.5 mm) that is difficult to measure except with the use of a microscope.

Glass particles were visible in soil and adhering to carrot tubers for treatments including and above the 0.25 t glass /ha treatment, the lowest glass application rate used in this trial. Similarly, glass particles were isolated from soil adhered to carrots at this application rate. This rate of glass application approximately corresponds to an MWOO application of 25 t/ha, which is higher than is currently allowed the 2014 NSW EPA Resource Recovery Order. Although the 0.25 t glass /ha treatment was the lowest application rate used in this trial, it is probable that there would still be visible glass in treatments with lower rates of glass application, although this was not tested. It should also be noted that because the experimental treatments were based on the amounts applied in the > 2mm fraction, thus aligning with current waste regulations pertaining to this material, there is also an additional amount of glass in the fraction < 2mm. This meant that total glass applied in the 0.25 t glass /ha (> 2mm fraction) exceeded 0.6 t total glass/ha.

The 0.25 t glass /ha application is 2.5 times higher than the maximum allowable rate allowed for broad acre agriculture, but less than that allowable under plantation and mine site rehabilitation scenarios. These calculations do not account for very fine glass (< 0.5 mm fraction) which could only be seen using microscopy and could not be isolated from amended soils.

Table 5: Calculated amounts of glass applied to soil with various applications of MWOO compared to current application limits. This table was constructed using MWOO analysis data and fractionation data collected from this trial. This assumes MWOO has an average total solids content (> 2mm) of 2%, one half of which is glass and that for every 1 kg glass in the > 2mm fraction, there is also 1.63 kg fine glass (< 2mm). Current application limits under the 2014 NSW EPA Resource Recovery Order are shown in italics. The application rate of MWOO equating to the glass treatments we used, where the appearance of glass on carrot tubers was first noted are also highlighted.

MWOO application rate	Solids > 2mm	Glass > 2mm	Glass < 2mm	Total glass added
t/ha	t	t	t	t
1	0.02	0.01	0.02	0.03
10	***0.2***	***0.1***	***0.16***	***0.26***
20	0.4	0.2	0.33	0.53
25	**0.5**	**0.25**	**0.41**	**0.66**
30	0.6	0.3	0.49	0.79
50	***1***	***0.5***	***0.82***	***1.32***
60	1.2	0.6	0.98	1.58
100	2	1	1.63	2.63
140	***2.8***	***1.4***	***2.28***	***3.68***
200	4	2	3.26	5.26
300	6	3	4.89	7.89
500	10	5	8.16	13.16
1000	20	10	16.32	26.32
1500	30	15	24.47	39.47
2000	40	20	32.63	52.63

Conclusions

Waste regulation currently permits applications of up to 3.5 t/ha of glass contaminant to mine sites, 0.75 t/ha to plantation forestry and non-contact agriculture and 0.15 t/ha for broad acre agricultural land.

The addition of physical contaminants to soils, such as glass, has the potential to harm human health through physical contact and may also cause damage to the soil habitat for growing crops (Terman and Mays, 1973) or soil biota (Stamatiadis and Dindal 1990). There is also the potential for physical contaminants to adversely affect grazing animals (Krause et.al. 1996; Chanie and Tesfaye, 2012), and so both International livestock health authorities, and bodies representing livestock production in Australia, have specific requirements for livestock producers, to prevent the occurrence of physical contaminants in livestock products.

Results from our model studies suggest that there are minimal effects of glass addition on earthworm behaviour, at MWOO application rates that are currently allowable. Similarly, we did not find any effect of glass application on legume nodulation for MWOO applications allowed under current legislation.

Visible glass was observed on the surface of all tubers harvested from glass amended soils. Glass was also isolated from soils adhered to tubers at harvest and in peel separated from these tubers. For proprietary reasons, we had limited access to quality assurance procedures and standards for food manufacturers based in Australia. However, the presence of glass in a harvested food product is not allowed under product Quality Assurance Standards used by at least one Australian retail food chain (Woolworths).

Glass was found on carrot tubers for a glass application rate of 0.25 t glass /ha which is equivalent to a MWOO application of 25 t/ha. This MWOO application rate exceeds current application guidelines for broad acre agriculture. Although the 0.25 t glass /ha treatment was the lowest application rate used in this trial, it is probable that there would still be visible glass in treatments with lower rates of glass application. It should also be noted that with each amount of glass applied to the soil in the > 2mm fraction, there is also an amount applied in the < 2mm fraction, and therefore, the total amount of glass applied in the 0.25 t glass /ha treatment, exceeded a total glass loading of 0.6 t/ha.

Our measurement of glass particles in soil and adhered to carrot tubers, do not account for very fine glass (< 0.5 mm fraction), which could only be seen using microscopy and could not be easily isolated and quantified in amended soils. There is some concern that fine (nano-sized) particles could adversely affect animal and human health (Hoet et.al. 2004).

Given that this model study was conducted under controlled conditions using glass-only amended soils, it is recommended that the impact of glass on tuber vegetables such as carrots be verified under field conditions where actual MWOO has been applied. At the same time, it should also be noted that there has been no assessment of the impact of glass particles on livestock health and production in grazing systems.

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Appendix 1. Properties of the Night Paddock Soils.

Location ID	**NB5**			
Site Location	Lower slope Northern corner			
Soil classification Isbell (1996)	Haplic Eutrophic Red chromosol			
	A1	A2	B21	B22
Depth (cm)	0-24	24-36	36-77	77-100
Boundary		clear	clear	gradual
Texture	CL	CL	L/M C	L/M C
FTG (span)	4	4	5 (>1.5)	5
Colour	7.5YR 3/2 dk bn	10YR4/4 dk yell bn	2.5YR 4/3 r bn	2.5YR 4/6 r
VC rating	1	5	5	5
structure	M	M	S	S
pH_C	5.64		6.36	
EC (dS/m)	0.09		0.05	
ECEC [cmol(+)/kg]	9.06		20.9	
Exch Al				
Exch Ca “	6.1		11	
Exch Mg “	1.8		9	
Exch Na “	0.057		0.33	
Exch K “	1.1		0.58	
ESP (%)	0.72		1.65	
Total P (%)	0.073		0.02	
Colwell P (mg/kg)	84		0.32	
Bray P (mg/kg)	22		0.18	
OC (%)	2.1		0.59	
Total N (%)	0.25		0.058	
Total As (mg/kg)	5.5		8.7	

Appendix 2. Average shoot dry weight and nodule scores for rhizobia effectiveness experiment. L.s.d. indicates statistical significance at p>0.05

TRT #	rate (t/)ha	rep	shoot dwt (g)	nodule score	lateral score
1	0	1	2.76	2.00	2.89
1	0	2	3.31	3.67	2.67
1	0	3	2.41	1.56	2.22
2	0.25	1	2.78	1.56	1.89
2	0.25	2	2.70	1.33	2.78
2	0.25	3	2.61	1.11	2.56
3	0.5	1	2.52	1.44	2.00
3	0.5	2	2.21	1.89	2.11
3	0.5	3	2.57	1.33	2.33
4	1	1	2.61	1.78	3.00
4	1	2	2.86	1.89	2.00
4	1	3	3.07	2.89	2.67
5	3	1	2.53	0.78	1.44
5	3	2	2.79	2.11	2.56
5	3	3	2.53	1.67	2.22
6	5	1	2.12	1.22	2.44
6	5	2	2.57	1.89	2.11
6	5	3	2.99	1.89	2.33
7	10	1	2.78	2.11	2.56
7	10	2	2.56	0.89	2.67
7	10	3	2.65	2.22	2.22
8	15	1	2.66	1.67	2.33
8	15	2	2.69	2.11	2.78
8	15	3	2.56	2.67	2.22
9	20	1	2.83	1.56	3.00
9	20	2	2.46	1.33	2.11
9	20	3	2.63	1.67	2.44
10	0	1	2.51	2.33	2.11
10	0	2	2.16	0.33	1.00
10	0	3	2.43	1.67	2.00
l.s.d.			***0.33***	***1.16***	***0.74***