25. Woolson, R. F., *Statistical Methods for the Analysis of Biomedical Data*, John Wiley, New York, 1987.
26. Knasmüller, S. *et al*., Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens. *Mutat. Res*., 1998, **402**, 185–202.
27. Jaeschke, H., McGill, M. R., Williams, C. D. and Ramachandran, A., Current issues with acetaminophen hepatotoxicity – a clinically relevant model to test the efficacy of natural products. *Life Sci*., 2011, **88**, 737–745.
28. Zhao, P., Kalhorn, T. F. and Slattery, J. T., Selective mitochondrial glutathione depletion by ethanol enhances acetaminophen toxicity in rat liver. *Hepatology*, 2002, **36**, 326–335.
29. James, L. P., Mayeux, P. R. and Hinson, J. A., Acetaminophen-induced hepatotoxicity. *Drug Metab. Dispos*., 2003, **31**(12), 1499–1506.
30. Jain, M., Kapadia, R., Jadeja, R. N., Thounaojam, M. C., Devkar, R. V. and Mishra, S. H., Cytotoxicity evaluation and hepatoprotective potential of bioassay guided fractions from *Feronia limmonia* Linn leaf. *Asian Pac. J. Trop. Biomed*., 2011, 443–447.
31. Peng, X. R., Liu, J. Q., Han, Z. H., Yuan, X. X., Luo, H. R. and Qiu, M. H., Protective effects of triterpenoids from *Ganoderma resinaceum* on H_2O_2-induced toxicity in HepG2 cells. *Food Chem*., 2013, **307**, 67–73.
32. Martin, M. A., Ramos, S., Mateos, R., Serrano, A. B. G., Pulido, M. I., Bravo, L. and Goya, L., Protection of human HepG2 cells against oxidative stress by cocoa phenolic extract. *J. Agric. Food Chem*., 2008, **56**, 7765–7772.
33. Farshori, N. N., Al-Sheddi, E. S., Al-Oqail, M. M., Hassan, W. H., Al-Khedhairy, A. A., Musarrat, J. and Siddiqui, M. A., Hepatoprotective potential of *Lavandula coronopifolia* extracts against ethanol induced oxidative stress-mediated cytotoxicity in HepG2 cells. *Toxicol. Ind. Health*, 2015, **31**(8), 727–737 (DOI: 10.1177/0748233713483188).
34. Mishra, J., Yousuf, A., Singh, R. D. and Aradhana, Phytochemical investigation and *in vitro* antioxidant potential of leaves of *Murraya koenigii*. *Int. J. Integr. Biol*., 2009, **9**, 171–174.
35. Rajeshkumar, N. V., Joy, K. L., Girija, K., Ramsewak, R. S., Nair, M. G. and Ramadasan, K., Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract. *J. Ethnopharmacol*., 2002, **81**, 17–22.
36. Syamasundar, K. V., Singh, B., Thakur, R. S., Husain, A., Kiso, Y. and Hikino, H., Antihepatotoxic principles of *Phyllanthus niruri* herbs. *J. Ethnopharmacol*., 1985, **14**, 41–44.
37. Lima, C. F., Fernandes-Ferreira, M. and Pereira-Wilson, C., Phenolic compounds protect HepG2 cells from oxidative damage: relevance of glutathione levels. *Life Sci*., 2006, **79**, 2056–2068.
38. Yang, S. Y., Hong, C. O., Lee, G. P., Kim, C. T. and Lee, K. W., The hepatoprotection of caffeic acid and rosmarinic acid, major compounds of *Perilla frutescens*, against t-BHP-induced oxidative liver damage. *Food Chem. Toxicol*., 2013, **55**, 92–99.
39. Naidu, V. G. M. *et al.*, Antioxidant, hepatoprotective and cytotoxic effects of icetexanes isolated from stem bark of *Premna tomentosa*. *Phytomedicine*, 2014, **21**(4), 497–505.
40. Pareek, A., Godavarthi, A. and Nagori, B. P. *In vitro* hepatoprotective activity of *Corchorus depressus* L. against CCl_4 induced toxicity in HepG2 cell line. *Pharmacogn. J*., 2013, **5**, 191–195.
41. Arumanayagam, S. and Arunmani, M., Hepatoprotective and antibacterial activity of *Lippia nodiflora* Linn. against lipopolysaccharides on HepG2 cells. *Pharmacogn. Mag*., 2015, **11**(41), 24–31.

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Toxicity study in mice fed with corn produced in soil containing tannery sludge vermicompost and irrigated with domestic wastewater

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Growing food in unconventional systems such as those using irrigation with domestic wastewater and the use of potentially toxic waste has generated resistance from producers and consumers. Here, we evaluate the possible physical and biochemical damage to Swiss mice fed for 13 weeks with corn produced in soil containing tannery sludge vermicompost and irrigated with wastewater from domestic sewage. The corn was offered as an additional food to standard rodent chow at a daily concentration of 15 g/kg of body mass. The results showed no changes in body weight of the animals during the experimental period. The consumption of grain and weight gain of the animals was stable. The total protein, albumin, globulin and alkaline phosphatase levels did not differ among experimental groups. In addition, macroscopic analysis of the liver of the animals showed no sign of injury or disorders. Thus, we preliminarily conclude that the maize produced in this way is innocuous to animals. However, further studies are needed to evaluate other variables not measured in the present study which can contribute to food security and the nutrition of the corn thus produced.

Keywords: Agro-industrial waste, animal models, toxicity, wastewater.

INDUSTRIAL processes and human activities generate waste that can be harmful to the environment and human health[1]. Such waste is a serious threat to the present quality of life[2] and is typified in the wastes generated from processing of bovine leather.

While these activities do generate significant profits and contribute to the economic and social development of a country, they also produce significant amounts of waste. This problem is intensified, especially when one considers that in many tanning industries waste/effluent

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Table 1. Experimental groups established in the present study

Experimental group	ID	Description
Control	G1	Composed of animals fed only standard rodent chow
Group corn commercial[a]	G2	Composed of animals fed standard rodent chow supplemented with commercial corn grain (Anchieta®). This was the second control group because the corn used here was commercially available.
Group chemical fertilization[b] – water supply[c]	G3	Composed of animals fed standard rodent chow supplemented with corn[f] grains produced by plants grown in soil with conventional chemical fertilizer and irrigated with water supply. This group was the third control group and used corn grown in the same soil and conditions of corn used for G4 and G5.
Group chemical fertilization[b] – domestic wastewater[d]	G4	Composed of animals fed standard rodent chow supplemented with corn[f] produced by plants grown in soil with conventional chemical fertilization, but irrigated with wastewater from sewage.
Tannery sludge vermicompost group[e]	G5	Composed of animals fed standard rodent chow supplemented with grain corn[f] produced by plants grown in soil containing tannery sludge vermicompost mixed with manure (20% of tannery sludge and 80% of cattle manure) plus phosphate fertilizer and irrigated with domestic wastewater.

ID, Identification of the experimental groups.

[a]The conditions for the cultivation of corn and seed variety were not identified in the packaging.

[b]The nitrogen, phosphorus and potassium (NPP) used in the cultivation of corn were calculated based on the nutritional needs of the crop, the nutrient concentrations in the soil (Oxisoil Typical) and yield expectation of 10 Mg per ha according to ref. 22. The NPP sources were urea (CH_4N_2O), superphosphate (P_2O_5) and potash (K_2O), respectively.

[c]Irrigation water used in the cultivation of corn offered in this experimental group was from the water supply system *Instituto Federal Goiano* (Urutaí, GO, Brazil) treated in a water treatment plant institution itself.

[d]Wastewater used for irrigation of corn offered in this experimental group was from a domestic wastewater stabilization pond located on the premises of Instituto Federal Goiano (Urutaí, GO, Brazil).

[e]The corn kernels were from the treatment with the highest productivity in the study of Malafaia[22].

[f]Cultivar LG 6036 (LG Sementes®).

produced is disposed of in an improper manner or put in 'dumps', landfills or industrial landfills (localized within the industrial land). This accumulation and concentration of potentially toxic material lead to a high risk of environmental contamination[3–7].

Fixing this problem can be a win-win situation. Indeed, there is active research on new ways to dispose and recover waste. Various studies have shown the benefits of tannery sludge in different agricultural crops such as sugarcane ratoon[8], conilon coffee[9], beans[10], lettuce[11,12], soybean[13], wheat, lettuce and radish[14] and maize[3,15–22]. Concurrently, there are great demands for the use of sewage wastewater in agriculture because it can act as a fertilizer and improve yield[23]. Several studies have shown the advantages of water in agriculture because it adds nutrients that are beneficial for plant growth[23,24–30].

Despite the evidence that both the waste produced in tanning industries and wastewater can be useful in agriculture, there is still resistance from farmers with few practical applications[23]. Part of this resistance is related to food and nutrition security linked to food produced in soils enriched with waste or potentially contaminated with wastewater sewage. These foods could then be risky.

Here, we evaluate the possible biochemical and weight damage to Swiss mice fed corn produced in soil containing tannery sludge vermicompost and irrigated with household wastewater.

We used female Swiss mice (50 days old) from the Biotério Central of the Universidade Federal de Goiás (Goiânia, GO, Brazil) that were maintained in the Biotério of the Laboratory of Biological Research, Instituto Federal Goiano (Urutaí, GO, Brazil). The animals were subjected to light/dark normal cycle (12/12 h) and kept in collective cages with a polycarbonate ventilated shelf (Alesco®) under controlled temperature (22–25°C).

The treatments consisted of grains offered as additional food to animals fed a standard rodent chow (Nuvilab CR1-Nuvital®), as described in Table 1. All the animals of the experimental group received rodent diet ad libitum, ensuring appropriate nutrition. Therefore, corn was an additional food to the standard diet. The grain was obtained from the work of Malafaia[22]. We used 50 Swiss mice and distributed them in five groups (n = 10 per group).

Tables 2 and 3 present the chemical and physicochemical characteristics of the soil, tannery sludge vermicompost and irrigation water used in corn farming.

Each day the animals received 15 g/kg body weight of corn as mentioned in the literature[31–34]. The amount of grain given to the animal's weight was measured daily for the offering of the selected grain concentration (15 g/kg body weight). Corn was kept in sterile containers with no processing or treatment.

We monitored the animals for 13 weeks. This was reasonable considering the lack of standard protocols for assessing food security and nutrition from non-conventional crops. This period has been used in previous studies designed to assess the safety of genetically modified subchronic food testing[35].

To evaluate possible adverse physical effects from the corn, we evaluated body mass (g) with results expressed in weekly averages. At the end of the experiment, we reported animal weight gain, grain consumption, as well as

serum total protein, globulin, albumin and alkaline phosphatase concentrations.

Serum total protein concentration was calculated using the biuret method utilizing the commercial kit Labtest Diagnostic SA®, Cat. 99 (Lagoa Santa, MG, Brazil). For assessment of serum albumin concentration, we used the bromocresol green method with the commercial kit Labtest Diagnostic SA®, Cat. 19 (Lagoa Santa, MG, Brazil). From the total protein and albumin concentrations, we calculated globulin concentration. For this, we used the following equation: globulin = total protein globulin – albumin. Alkaline phosphatase measurement was done using a method described by Bowers and McComb with the commercial kit Labtest Diagnostic SA®, Cat. 79 (Lagoa Santa, MG, Brazil). For these experiments, the animals were fasted for at least 12 h and 2 ml of blood was collected via the brachial artery. The blood was placed in 5 ml tubes containing anticoagulant.

Prior to blood collection, the animals were anesthetized with tribromoethanol 2% (1 ml/100 g, i.p.) followed by cervical dislocation. The livers were removed for macroscopic analysis of possible changes in them. The livers were evaluated for colour as microscopic capsular cutting surfaces. We noted the presence or absence of nodulation (colour, quantity, size and distribution) or any sign of liver damage that could be related to toxicity.

Initially, data were subjected to normality test of Anderson–Darling, Kolmogorov-Smirnov, Cramér-von Mises, Kuiper, Watson and Lilliefors, followed by the homogeneity test using Levene variance (ASSISTAT software, version 7.7 beta (copy distributed free)). All data were normally distributed a 5% probability ($P > 0.15$). Statistical differences were calculated for comparison analysis. The body mass data were subjected to analysis of variance (ANOVA) for repeated measures ANOVA followed by the Duncan test at 5% probability. For data on biochemical assessments, we used the simple one-way ANOVA followed by Duncan test at 5% probability.

The methodology of this study was considered consistent with the ethical principles for animal experimentation and approved by the Committee on Animal Research and Ethics of the Instituto Federal Goiano (GO, Brazil) (protocol no. 003/2014).

We observed body mass for 13 weeks (Figure 1 *a*). This period was chosen due to lack of standard protocols for assessing food security and nutrition from non-conventional crops. Thus, we based our study on those that assessed the toxicity of substances, tea, drinks in

Table 2. Main characteristics of the initial soil and tannery sludge vermicomposting used in the present study. Urutaí, GO, Brazil, 2014

	Results	
Diversify	Ground	Vermicomposting (Lc20)*
pH ($CaCl_2$)	5.30	8.8
N (%)	0.11	1.5
P (Melich – mg dm^{-3})	5.00	700.0
K (mg dm^{-3})	240.00	18,000.0
Ca (cmolc dm^{-3})	2.60	14.0
Mg (cmolc dm^{-3})	0.80	18.0
Ca + Mg (cmolc dm^{-3})	3.40	32.0
Al (cmolc dm^{-3})	0.00	0.0
H + Al (cmolc dm^{-3})	2.20	0.0
CTC (cmolc dm^{-3})	6.20	82.4
Na (mg dm^{-3})	8.00	1.000.0
Cu (mg dm^{-3})	2.50	5.0
Fe (mg dm^{-3})	63.00	244.0
Mn (mg dm^{-3})	47.00	68.0
Zn (mg dm^{-3})	4.40	36.0
Organic matter (%)	2.30	29.9
Sat Al (%)	0.00	0.0
Sat base (%)	65.00	100.0
Ca/Mg (%)	3.30	0.8
Ca/CTC (%)	42.00	17.0
Mg/CTC (%)	13.00	22.0
K/CTC (%)	10.00	56.0
H + Al/CTC (%)	35.00	0.0
Clay (%)	27.00	–
Silt (%)	15.00	–
Sand (%)	58.00	–
Electrical conductivity (μS cm^{-3})	184.00	1.170,0
Total organic carbon (%)	1.30	17.3
Particle density (g cm^{-3})	2.45	–
Cr (mg dm^{-3})	<5.00	<5.0

*Vermicomposting (Lc20): Sludge vermicompost tannery made up of 20% type liming tannery sludge and 80% cattle manure.

Table 3. Physical, chemical and physico-chemical characterization of the irrigation water used in the present study

Parameters	Supply water*	Wastewater*
pH at 25°C	7.20	6.75
Turbidity (UNT)	8.00	931.00
Dissolved Fe (mg l^{-1})	0.68	1.77
Total N (mg l^{-1})	3.00	54.95
Organic N	ND	3.95
Ammoniacal N (mg l^{-1})	0.17	43.00
Nitrate (mg l^{-1})	0.30	8.00
Electric conductivity at 25°C (μs cm^{-1})	49.10	738.00
Total P (mg l^{-1})	0.22	10.29
Orthophosphate (mg l^{-1})	0.50	23.60
BOD (mg l^{-1})	0.70	733.33
Total solids (mg l^{-1})	80.00	1,790.00
Dissolved Cu (mg l^{-1})	1.00	1.00
Zn (mg l^{-1})	0.03	0.07
Na (mg l^{-1})	5.25	83.50
Dissolved Mn (mg l^{-1})	1.20	2.20
Dissolved Mg (mg l^{-1})	2.43	4.86
Ca (mg l^{-1})	4.00	20.04
S (mg l^{-1})	2.00	2.66
K (mg l^{-1})	1.64	26.00
TOC (mg l^{-1})	18.36	46.00

ND, Parameter not analysed. BOD, Biochemical oxygen demand; TOC, Total organic carbon.
*Values shown are the mean of three samples collected during the experimental period.

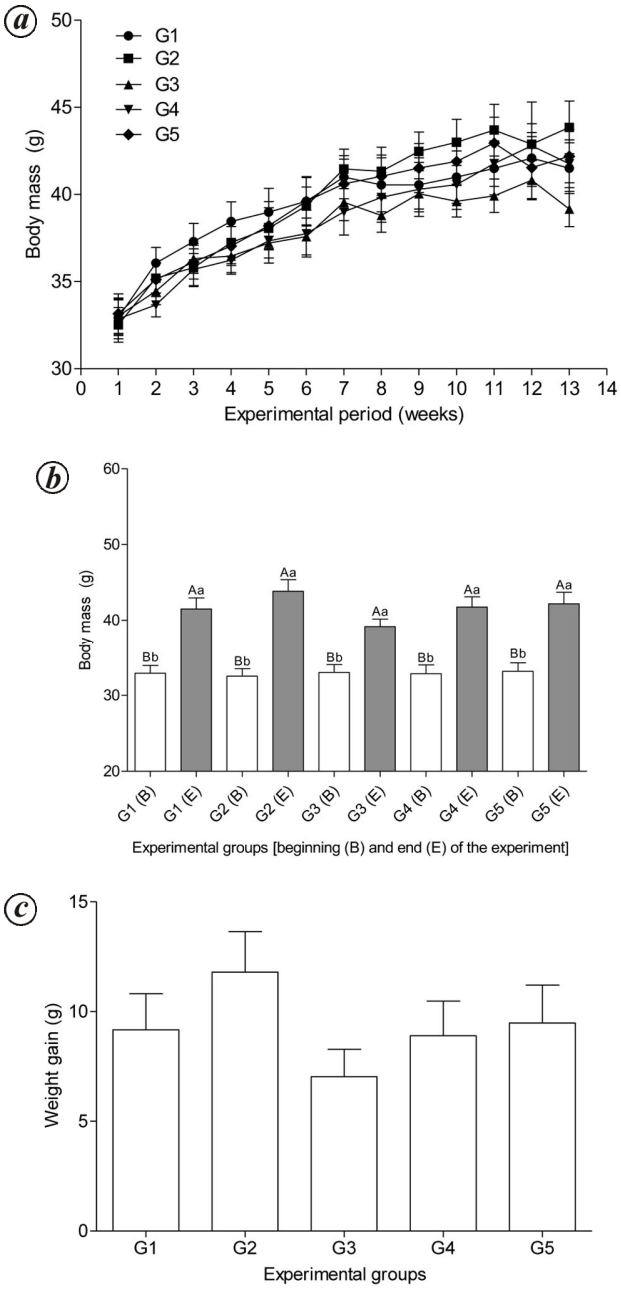

Figure 1. ***a***, Change in body weight throughout the experiment; ***b***, initial and final mass; ***c***, weight gain in Swiss mice fed with corn grain produced under different conditions, as an additional food to the standard rodent diet. In (***a***) the points on the curve represent the means ± SEM of the groups at each week of the trial. The body mass data were analysed by analysis of variance for repeated measures ANOVA followed by the Duncan test at 5% probability. In (***b***) and (***c***), the bars represent the mean ± SEM. We used one way ANOVA at the 5% probability level. In (***b***) different lowercase letters indicate statistical differences between initial and final values of each group with Student's *t* test at 5% probability. Equal capital letters indicate that there is no statistical difference between groups by one-way ANOVA at 5% probability while comparing them from the beginning to the end of the experiment. In (***c***) we show one-way ANOVA at 5% probability. G1, Control group; G2, Group commercial corn; G3, Chemical fertilizer group – water supply; G4, Chemical fertilizer group – wastewater; and G5, Tannery sludge vermicompost group (see Table 1 for details). $n = 10$ for G1, G4 and G5 and $n = 9$ for G2 and G3. Two animals of G2 and G3 died during the experiment due to unknown causes.

general or conventionally produced food[36–42]. These studies in general have considered that 13 weeks correspond to sub-chronic exposure.

All groups gained weight at the end of the experiment compared to their initial mass (Figure 1 *b*). This is due to the growth and natural development of the animals. There was no difference in the amount of weight gained between the different groups (Figure 1 *c*) ($F_{(4,43)} = 1.027$, $P = 0.404$). These results show that corn consumption did not affect mouse growth and development.

To our knowledge, there have been no previous studies on the effects of corn grown in soil containing vermicompost and domestic wastewater on mouse health. Júnior *et al.*[43] have shown that weight gain is an early indicator of toxicity. Several studies have reported that weight parameters in animal studies can assess the effect of substances in the body for medical, food and environmental toxicology[44–48].

There were no differences between the groups in terms of maize consumption (Figure 2) ($F_{(4,45)} = 0.597$, $P = 0.668$). This was expected because the amount of grain supplied depended on the body weight of the animals. This did not differ during the beginning, middle or end of the experimental period.

There was no difference between the experimental groups for total protein ($F_{(4,43)} = 0.487$, $P = 0.744$), albumin ($F_{(4,43)} = 2.248$, $P = 0.079$), globulin ($F_{(4,43)} = 0.784$, $P = 0.541$) (Figure 3 *a*) and the enzyme alkaline phosphatase ($F_{(4,40)} = 2.780$, $P = 0.102$) (Figure 3 *b*). These results show that the additional intake of corn did not change these biochemical parameters.

These results are similar to recent reports on Swiss mice[49,50] indicating that corn supplements do not change these parameters. It is known, for example, that total protein levels provide insight into the health of an organism[51].

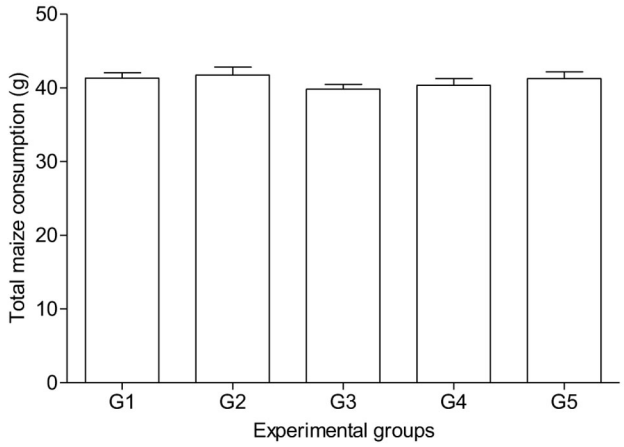

Figure 2. Total consumption of grains during the experimental period. The bars represent the mean ± SEM of independent experiment. We used one-way ANOVA at 5% probability level. G1–G5, same as in Figure 1. $n = 10$ for G1, G4 and G5 and $n = 9$ for G2 and G3. Two animals of G2 and G3 died during the experiment due to unknown causes.

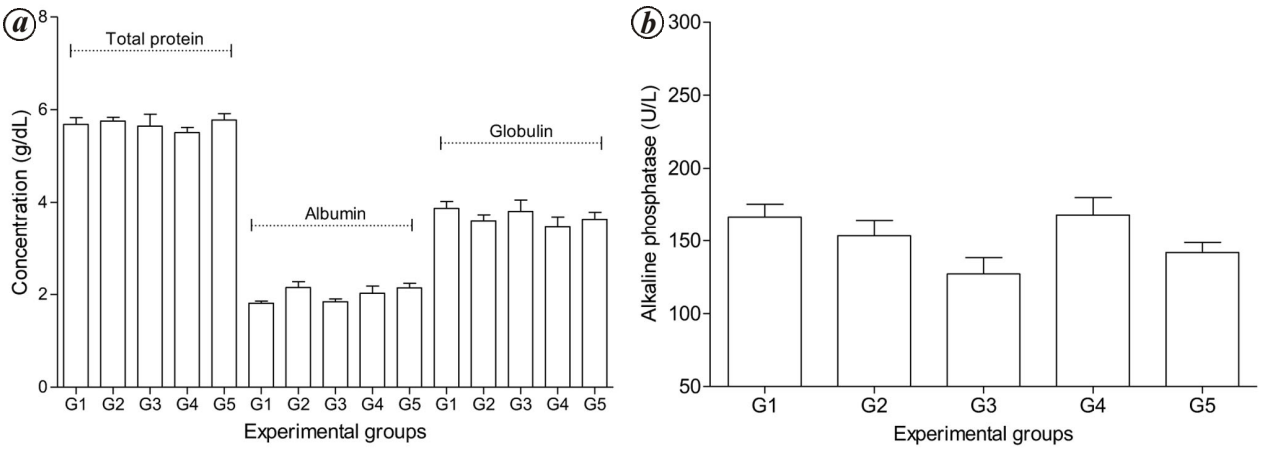

Figure 3. ***a***, Serum concentrations of total protein, albumin, globulin; ***b***, concentration of the enzyme alkaline phosphatase from Swiss mice fed with corn grown under different conditions. The bars represent the mean ± SEM of independent experiment. We used one way ANOVA at the 5% probability level. G1–G5, same as in Figure 1. $n = 10$ for G1, G4 and G5 and $n = 9$ for G2 and G3. Two animals of G2 and G3 died during the experiment due to unknown causes. $n = 8$ for G1 (two alkaline phosphatase outliers have been removed), $n = 9$ for the G2 and G3 and $n = 10$ for G4 and G5 groups.

Albumin and alkaline phosphatase enzyme are biomarkers of liver injury[52]. Albumin is the most abundant protein in the plasma and extracellular fluids[53], and is one of the most frequently used variables to compose prognostic indexes. It is the best single marker of complications[54]. Hypoalbuminuria may be correlated to chronic liver damage[52] as well as kidney damage.

Alkaline phosphatase and in particular phosphohydrolase is an enzyme found in many tissues with the highest concentrations in liver, biliary tract epithelium and bone[52]. Generally, any active liver disease may increase the phosphatase values, but drastic increases in enzyme levels occur in the case of biliary tract obstruction that may be induced by drugs or toxic substances in water or food intake. Therefore, this enzyme may be an important marker of activity in the plasma membrane and endoplasmic reticulum during pharmacological treatments and in some pathological conditions. Increases in serum alkaline phosphatase activity can occur during cholestasis as well as intra- and extrahepatically via drugs or hormones or by osteoblastic hyperactivity and necrotic processes[55]. Therefore, our data indicate that animals of different groups show no obvious biochemical signs of liver toxicity.

Macroscopic analysis of the liver of animals showed no signs of liver injury. Qualitative analysis revealed that the livers of the animals were healthy and normal.

Therefore, we can conclude that despite the tannery sludge vermicompost and domestic wastewater containing high concentrations of various elements or increased physical and chemical indicators (Tables 2 and 3), they caused no biological effects that indicate toxicity in animals. Although we have not studied the bioavailability of the elements in soil and in plants, in particular, probably those elements were not in bioavailable forms for maize plants, preventing their absorption and, consequently, their translocation to the corn grains.

Studies in the literature with genetically modified (GM) foods point to the concern of the effects – it is not fully known – that the introduction of new genes in organisms serve as food. Results involving the supply of GM laboratory animal diets have been troublesome, as discussed by Smith[56].

Studies have found that the abnormal and damaged cells of the small intestine of mice fed with transgenic potatoes carriers of bacterial gene that produces insecticidal *Bt* (*Bacillus thuringiensis*)[57]. Stomach intestinal epithelial cells from mice fed with potato lectin *Galanthus nivalis* (GNA), which is a type of insecticide[58], also had increased growth. These results are important because cell proliferation can be a precursor to cancer.

With regard to liver damage, different authors have reported different types of damage depending on the feed[59–62]. This requires further studies given the complexity of the organic system and the variety of treatments offered to animals.

This preliminary study offers preliminary evidence that the consumption of maize produced with tannery sludge worm compost and sewage wastewater does not cause toxicity relative to controls. Thus, this approach may be an important food supply that also reduces agro-industrial waste and increases water reuse.

However, it is essential to consider that this study is not exhaustive and further research is needed to safely assess the risks of corn meal produced under experimental conditions of this study. Future work will use different concentrations of maize, alone or as a food supplement, different feeding periods as well as other animal models and strains.

1. Kraemer, M. E. P., A questão ambiental e os resíduos industriais; http://www.amda.org.br/imgs/up/Artigo_25.pdf (accessed on 14 November 2014).
2. Silva, A. S. S., Souza, J. G. and Leal, A. C., Qualidade de vida e meio ambiente: experiência de consolidação de indicadores de

sustentabilidade em espaço urbano. *Sustentabilidade Debate*, 2012, **3**, 177–196.
3. Konrad, E. E. and Castilhos, D. D., Alterações químicas do solo e crescimento do milho decorrentes da adição de lodos de curtume. *R. Bras. Ci. Solo*, 2002, **26**, 257–265.
4. Pacheco, J. W. F. Curtumes: Série P + L, 2005; http://www.cetesb.sp.gov.br (accessed on 5 January 2013).
5. Pinheiro, F. G. R., Avaliação do Potencial Poluidor de curtumes do Distrito Industrial de Icoaraci e influência sobre os recursos hídricos locais. 2005. 140f. Dissertation (Master in Geology)–Centro de Geociências. Universidade Federal do Pará, Belém, Brazil, 2005.
6. Ganem, R. S., Curtumes: aspectos ambientais, 2007; http://bd.camara.gov.br/bd/bitstream/handle/bdcamara/1281/curtumes_aspectos_senna.pdf?sequence=1 (accessed on 20 May 2013).
7. Godecke, M. V., Rodrigues, M. A. S. and Naime, R. H., Resíduos de curtume: estudo das tendências de pesquisa. *REGET*, 2012, **7**, 1357–1378.
8. Batista, M. M. and Alovisi, A. M. T., Alterações de atributos químicos do solo e rendimento da cana soca pela utilização de lodo de curtume. *An. Prod. Inic. Cien. Disc.*, 2010, **13**, 387–396.
9. Berilli, S. S., Quiuqui, J. P. C., Rembinski, J., Salla, P. H. H., Berilli, A. P. C. G. and Louzada, J. M., Utilização de lodo de curtume como substrato alternativo para produção de mudas de café conilon. *Coffe Sci.*, 2014, **9**, 472–479.
10. Prado, A. K. and Cunha, M. E. T., Efeito da aplicação de lodo de esgoto e curtume nas características físico-químicas do solo e na absorção de nitrogênio por feijoeiro (*Phaseolus vulgaris* L.). *UNOPAR Cient. Exatas Tecnol.*, 2011, **10**, 34–41.
11. Aquino-Neto, V. A. and Camargo, O. A., Crescimento e acúmulo de crômio em alface cultivada em dois latossolos tratados com $CrCl_3$ e resíduos de curtume. *R. Bras. Ci. Solo*, 2000, **24**, 225–235.
12. Bastos, N. S., Merizio, T. and Araújo, F. F., Desenvolvimento de mudas de alface em substrato comercial enriquecido com lodo de curtume. *Colloquium Exactarum*, 2011, **3**, 18–21.
13. Costa, C. N., Castilhos, D. D., Castilhos, R. M. V., Konrad, E. E., Passionoto, C. C. and Rodrigues, C. G., Efeito de adição de lodo de curtume sobre as alterações químicas do solo, rendimento de matéria seca e absorção de nutrientes em soja. *Rev. Bras. Agroci.*, 2001, **7**, 189–191.
14. Castilhos, D. D., Tedesco, M. J. and Vidor, C., Rendimentos de culturas e alterações químicas do solo tratado com resíduos de curtume e crômio hexavalente. *R. Bras. Ci. Solo*, 2002, **26**, 2002.
15. Borges, J. D., Efeitos do lodo de curtume nas culturas do milho (*Zea mays* L.) e do capim braquiarão [*Brachiaria brizantha* (Hochst ex A. Rick) Sapf.] cultivar Marandu em Latossolo vermelho-amarelo. 2003. 244 f. Thesis (Doctor in Agronomy)–Escola de Agronomia, Universidade Federal de Goiás, Goiânia, Brazil, 2003.
16. Ferreira, A. S., Camargo, F. A. O., Tedesco, M. J. and Bissani, C. A., Alterações de atributos químicos e biológicos de solo e rendimento de milho e soja pela utilização de resíduos de curtume e carbonífero. *R. Bras. Ci. Solo*, 2003, **27**, 755–763.
17. Souza, E. R. B. *et al.*, Teores de metais tóxicos nas folhas de plantas de milho fertilizadas com lodo de curtume. *Pesq. Agropec. Trop.*, 2005, **35**, 117–122.
18. Da Silva, A. S., Laime, E. M., Souza, E. N., Souto-Filho, L. T. and Oliveira, S. C. D. E. S., Caracterização de plantas de milho na absorção de nutrientes e metais pesados após submissão à irrigação com efluentes. *Rev. Educ. Agric. Superior*, 2012, **27**, 141–145.
19. Borges, J. D. *et al.*, Teores de micronutrientes nas folhas de milho fertilizadas com lodo de curtume. *Biosci. J.*, 2007, **23**, 1–6.
20. Araújo, F. F., Tititan, C. S., Pereira, H. C. and Caetano-Júnior, O., Desenvolvimento do milho e fertilidade do solo após aplicação de lodo de curtume e fosforita. *Rev. Bras. Eng. Agríc. Ambient.*, 2008, **12**, 507–511.
21. Araújo, F. F., Disponibilização de fósforo, correção do solo, teores foliares e rendimento de milho após a incorporação de fosfatos e lodo de curtume natural e compostado. *Acta Sci. Agron.*, 2011, **33**, 355–360.
22. Malafaia, G., Aproveitamento de lodo de curtume e uso de água residuária de esgoto doméstico na cultura do milho (*Zea mays* L.). 2015. 230 f. Thesis (Doctor in Agronomy)–Escola de Agronomia, Universidade Federal de Goiás, Goiânia, Brazil, 2015.
23. Leal, R. M. P., Fonseca, A. F., Herpin, U. and Melfi, A. J., Agricultural utilization of treated sewage effluent: experience from Brazil. *Isr. J. Plant. Sci.*, 2011, **59**, 235–248.
24. Fonseca, A. F., Melfi, A. J. and Montes, C. R., Maize growth and changes in soil fertility after irrigation with treated sewage effluent. I. Plant dry matter yield and soil nitrogen and phosphorus availability. *Commun. Soil Sci. Plant Anal.*, 2005, **36**, 1965–1981.
25. Fonseca, A. F., Melfi, A. J. and Montes, C. R., Maize growth and changes in soil fertility after irrigation with treated sewage effluent. II. Soil acidity, exchangeable cations, and sulfur, boron, and heavy metals availability. *Commun. Soil Sci. Plant Anal.*, 2005, **36**, 1983–2003.
26. Fonseca, A. F., Herpin, U., Paula, A. M., Victoria, R. L. and Melfi, A. J., Agricultural use of treated sewage effluents: agronomic and environmental implications and perspectives for Brazil. *Sci. Agric.*, 2007, **64**, 194–209.
27. Andrade-Filho, J. A., Sousa, O. N., Sias, N. S., Nascimento, I. B., Medeiros, J. F. and Cosme, C. R., Atributos químicos de solo fertirrigado com água residuária no semiárido brasileiro. *Irriga*, 2013, **18**, 661–674.
28. Bonini, M. A., Sato, L. M., Bastos, R. G. and Souza, C. F., Alterações nos atributos químicos e físicos de um Latossolo Vermelho irrigado com água residuária e vinhaça. *Rev. Bioc.*, 2014, **20**, 56–63.
29. Silva, L. L., Carvalho, C. M., Souza, R. P. F., Feitosa, H. O., Feitosa, S. O. and Filho, R. R. G., Utilização de efluentes domésticos no crescimento da pimenta (*Capsicum chinense*), cultivar tequila bode vermelha. *Rev. AGROTEC*, 2011, **35**, 121–133.
30. Monteiro, D. R., Silva, T. T. S., Silva, L. V. B., Lima, V. L. A., Santos, C. L. M. and Pearson, H. W., Efeito da aplicação de efluente doméstico tratado nos teores de micronutrients no solo. *Irriga*, 2014, 40–46.
31. Momma, K. *et al.*, Quality and safety evaluation of genetically engineered rice with soybean glycinin: analyses of the grain composition and digestibility of glycinin in transgenic rice. *Biosci. Biotechnol. Biochem.*, 1999, **63**, 314–318.
32. Momma, K. *et al.*, Safety assessment of rice genetically modified with soybean glycinin by feeding studies on rats. *Biosci. Biotechnol. Biochem.*, 2000, **64**, 1881–1886.
33. Momma, K., Hashimoto, W., Utsumi, S. and Murata, K., Safety assessment of genetically modified rice with soybean glycinin. In *Molecular Methods of Plant Analysis* (eds Jackson, J. F., Linskens H. F. and Inman, R. B.), Springer-Verlag, Berlin, 2002, pp. 139–151.
34. Chen, Z. L. *et al.*, Safety assessment for genetically modified sweet pepper and tomato. *Toxicology*, 2003, **188**, 297-307.
35. Venzke, J. G., Segurança alimentar de milho geneticamente modificando contend o gene cry Ab de *Bacillus thrungiensis*. Dissertation (Master) – Universidade Federal de Pelotas, Pelotas, Brazil, 2006.
36. Park, H. S. *et al.*, A 90-day study of sub-chronic oral toxicity of 20 nm positively charged zinc oxide nanoparticles in Sprague Dawley rats. *Int. J. Nanomed.*, 2014, **15**, 93–107.
37. McCain, W. C., Crouse, L. C., Bazar, M. A., Roszell, L. E., Leach, G. J., Middleton, J. R. and Reddy, G., Subchronic oral toxicity of sodium tungstate in Sprague–Dawley rats. *Int. J. Toxicol.*, 2015, **1**, 1–10.
38. Sur, T. K., Chatterjee, S., Hazra, A. K., Pradhan, R. and Chowdhury, S., Acute and sub-chronic oral toxicity study of black tea in rodents. *Indian J. Pharmacol.*, 2015, **47**, 167–172.

39. Akindele, A. J., Unachukwu, E. G. and Osiagwu, D. D., 90 days toxicological assessment of hydroethanolic leaf extract of *Ipomoea asarifolia* (Desr.) Roem. and Schult. (Convolvulaceae) in rats. *J. Ethnopharmacol.*, 2015, **S0378–8741**, 196–198.
40. Li, Y. *et al.*, Acute and sub-chronic toxicity of glucose–cysteine Maillard reaction products in Sprague–Dawley rats. *Food Chem. Toxicol.*, 2015, **80**, 271–276.
41. Wang, X. *et al.*, Safety assessment of aditoprim acute, subchronic toxicity and mutagenicity studies. *J. Appl. Toxicol*., 2015, **1**, 1–12.
42. Lin, H. T. *et al.*, Repeated dose 90-day feeding study of whole fruits of genetically modified papaya resistant to *Papaya Ringspot Virus* in rats. *J. Agric. Food Chem.*, 2015, **63**, 1286–1292.
43. Júnior, H. B. P., Borges, L. M., Sousa, L. A. D., Cunha, L. C., Júnior, R. S., Melo, D. F. A. and Pereira, M. E., Avaliação da toxicidade aguda do extrato hexânico de frutos de *Melia azedarach* (Meliaceae) em camundongos. *Ciênc. Anim. Bras.*, 2012, **13**, 512–519.
44. Olson, J. A., Schwartz, J. A., Hahka, D., Nguyen, N., Bunch, T., Jensen, G. M. and Adler-Moore, J. P., Toxicity and efficacy differences between liposomal amphotericin B formulations in uninfected and *Aspergillus fumigatus* infected mice. *Med. Mycol.*, 2015, **53**, 107–118.
45. Bhaskar, R. and Mohanty, B., Pesticides in mixture disrupt metabolic regulation: *in silico* and *in vivo* analysis of cumulative toxicity of mancozeb and imidacloprid on body weight of mice. *Gen. Comp. Endocrinol.*, 2014, **205**, 2226–2234.
46. Lu, L. *et al.*, Safety assessment of the fermented *Phylloporia ribis* (*Lonicera japonica* Thunb.) mycelia by oral acute toxicity study in mice and 90-day feeding study in rats. *Food Chem. Toxicol*., 2014, **69**, 18–24.
47. Mukerji, P., Era, J. C., Buck, R. C. and O'Connor, J. C., Oral repeated-dose systemic and reproductive toxicity of 6 : 2 fluorotelomer alcohol in mice. *Toxicol. Rep*., 2015, **2**, 130–143.
48. Das, K. P. *et al.*, Developmental toxicity of perfluorononanoic acid in mice. *Reproduc. Toxicol.*, 2015, **51**, 133–144.
49. Costa, J. P. *et al.*, Avaliação da toxicidade aguda e das alterações histopatológicas em camundongos tratados com fitol. *Rev. Ciênc. Farm. Básica Apl.*, 2012, **33**, 421–428.
50. Castello-Branco, A. C. S. C., Diniz, M. F. F. M., Almeida, R. N., Santos, H. B. S., Oliveira, K. M., Ramalho, J. A. and Dantas, J. G., Parâmetros Bioquímicos e Hematológicos de Ratos Wistar e Camundongos Swiss do Biotério Professor Thomas George. *Rev. Bras. Ciênc. Saúde*, 2011, **15**, 209–214.
51. Estrela, A. D. C., Lemes, C. G. C., Guimarães, A. T. B. and Malafaia, G., Effects of short-term malnutrition in rats. *Sci. Plena*, 2014, **10**, 1–13.
52. Jesus, G. C., Sousa, H. H. B. A. and Barcelos, R. S. S., Principais patologias e biomarcadores das alterações hepáticas. *Estudos*, 2014, **41**, 525–537.
53. Bottoni, A., Oliveira, G. P. C., Ferrini, M. T. and Waitzberg, D. L. Avaliação nutricional: exames laboratoriais. In Nutrição oral, enteral e parenteral na prática clínica (ed. Waitzberg, D. L.), Atheneu, São Paulo, 2001, pp. 279–294.
54. Smith, L. C. and Mullen, J. L., Nutritional assessment and indications for nutritional support. *Surg. Clin. North Am*., 1991, **71**, 449–457.
55. Wright, P. J., Leathwood, P. D. and Plummer, D. T., Enzymes in rat urine: alkaline phosphatase. *Enzymologia*, 1972, **42**, 317–327.
56. Smith, J. M., *Seeds of Deception. Exposing Industry and Government Lies About the Safety of the Genetically Engineered Foods*, Chelsea Green Publishing, Fairfield, UK, 2003, 6th edn.
57. Nagui, H. F. and Adel, K. E., Fine structural changes in the ileum of mice fed on endotoxin treated potatoes and transgenic potatoes. *Nat. Toxins*, 1998, **6**, 219-233.
58. Stanley, W. B. E. and Pusztai, A., Effect of diet containing genetically modified patotoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet*, 1999, **354**, 1353–1354.
59. Malatesta, M., Caporaloni, C., Rossi, L., Battistelli, S., Rocchi, M. B. L., Tonucci, M. and Gazzanelli, G., Ultrastructural analysis of pancreatic acinar cells from mice fed on genetically modified soybean. *J. Anat.*, 2002, **201**, 409–415.
60. Malatesta, M., Baldelli, B., Battistelli, S., Tiberi, C., Manuali, E. and Miggiogera, M., Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean. *Eur. J. Histochem*., 2005, **49**, 237–242.
61. Séralini, G. R. *et al.*, RETRACTED: Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food Chem. Toxicol.*, 2012, **50**, 4221–4231; http://www.sciencedirect.com/science/article/pii/S0278691512005637
62. Malatesta, M., Boraldi, F., Annovi, G., Baldelli, B., Battistelli, S., Biggiogera, M. and Quaglino, D., A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. *Histochem. Cell. Biol.*, 2008, **130**, 967–977.

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Interventions to reduce drudgery of workers in the traditional method of harvesting Makhana (*Euryale ferox* salisb.) seeds from ponds

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Makhana (*Euryale ferox* salisb.) is a seed produced from an aquatic crop, which normally grows in water bodies like ponds. In the traditional way of harvesting, a worker goes deep into the pond, lies down, holds his breath and drags the mud with both hands towards a bamboo pole called 'kaara', which is later sieved using a bamboo screen called 'ganjaa'. During this operation mud enters into the ears, eyes, nose and mouth of the worker. Also, the workers are affected by skin-related diseases due to unhygienic working environment. Therefore, an intervention was made and an improved system was developed which consists of a floating platform providing support to a 10 l cylinder having compressed air, 10 m hose pipe with regulator and a mini diving kit having suit with cap, mask and content guage. A comparative study was conducted

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