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Biofertilization of Tifton 85 with Sludge from Sewage Treatment Station of Whey Industry

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The high concentration of N, P and other components present in sludge from wastewater treatment plants make it economically interesting the application in agricultural crop, as soil biofertilizer. The objective of this study was to evaluate the influence of the application of biological sludge, from an effluent treatment plant, in Tifton 85 crop, observing the performance of the sludge as biofertilizer and, finally, evaluating the bioaccumulation of nutrients and metals in the leaf tissue of plants biofertilized with the sludge. The experiment was carried out in completely randomized design

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(CRD), with application of five doses of dehydrated sludge, being the applied doses of 0, 10, 20, 30 and 40 m³ ha^{-1}, in six replicates. After 47 days of application of sludge, the forages were cut to 12 cm from soil, stored and dried, for further determination of dry mass and bioaccumulated contents of P, K, Ca, Mg, Cu, Zn, Fe, Mn, Pb and Cr. The application of sludge to soil did not present negative influences on the development of Tifton 85 forage, having contributed significantly to the increase of N, P and CP. However, at sludge doses above 50 mg kg^{-1}, Mn leaf contents reduced Fe absorption by plants. There was no significant absorption of metals such as Cr, Cd and Pb by the plant.

Keywords: Biofertilizer; organic fertilizer; agroindustrial waste; industrial sludge; sustainability.

1. INTRODUCTION

Almost all water used in human activities is contaminated in some way, leaving the water resources prone to contamination, the hydrological cycle makes the water used in economic activities to return to the environment, sometimes contaminated with chemical agents, organic matter and nutrients in high concentrations, what can conduct to eutrophication of water bodies [1].

The vast use of water, both in the production of milk derivatives and in the hygiene of producing equipment, makes the dairy industries fall into a list of potential polluters [2]. The high level of organic matter makes necessary previous treatment before throwing this effluent in the water bodies [3].

According to [4], liquid effluents generated in dairy production processes have high levels of organic matter, fats, suspended solids and nutrients, and are considered the main source of pollution of these industries. In a significant part of the dairy industry, the biological method is used as a treatment of effluents to maximize the removal of organic matter, using this method, the formation of sludge occurs [5].

The biological sludge is composed by microbial biomass itself and must be constantly removed from the system in order to avoid accumulation and possible biomass drag with the treated effluent [6]. The high concentration of nitrogen, phosphorus and other components make the sludge interesting for application on soil, in plant cultivation [7].

In cultivation of agricultural crops, however, the application should be well planned as it can transfer toxic agents to the soil. Several studies have demonstrated that the addition of sludge improves crop cultivation in several aspects, such as nutrient supply and absorption [8], an improvement in aerial/root dry mass ratio [9] and increased productivity [10].

Several studies have reported the use of biological sludge from treatment plants and their use in biofertilization of agricultural crops, such as: Use of biosolids in maize cultivation [8], oats [11] and *Eragrostis curvula* [12]. The milk industry sludge was specifically used [13] in radish production. However, the literature is deficient when it comes to the use of biological sludge from treatment plants of whey industry for biofertilization of agricultural crops.

It should also be noted that Brazilian and Paraná legislation is null and void as to the standardization of disposal of such solid waste (sludge from the treatment plant of the whey industry) in soil for agricultural purposes.

Considering the aforementioned, the present study research the influence of the application of sludge from the treatment plant of whey industry in Tifton 85, which have great ability to adapt to different environments, good production characteristics and rapid recovery after cutting, its use is quite widespread as a food for animal feed and has shown promise for plant recomposition in degraded areas, presenting significant tolerance to heavy metals.

The aim of this study was to evaluate the influence of the application of biological sludge from an effluent treatment plant of whey industry in Tifton 85 forage, to observe the performance of the sludge as a biofertilizer through the development of vegetal dry mass and to evaluate the bioaccumulation of nutrients and metals in the foliar tissue of plants.

2. MATERIALS AND METHODS

The experiment was carried out at the Pontifical Catholic University of Paraná (PUCPR), Toledo campus, located in the western region of Paraná state (24º 42 '49 "S and 53º 44' 35" W). The experiment was conducted in the period from May to July 2015, in a greenhouse with two irrigation periods of 30 minutes (7:00 a.m. and 7:00 p.m.).

In the soil characterization, the determination of toxic metals (Cr, Pb and Cd) by means of flame atomic absorption spectrometry (FAAS) [14], performed after nitro-percloric digestion [15], the other analyzes were performed after Mehlich extraction and sulfuric digestion [16]. Before sludge application, the chemical characteristics of the soil used in the experiment was evaluated and the results showed that: pH was 6.69, organic matter was 41.01 g dm^{-3}, P was 216.28 mg dm^{-3}, Al^{3+} was not identified, K, Ca, Mg was 1.34 $cmol_c$ m^{-3}, 11.03 $cmol_c$ m^{-3}, and 15.92 $cmol_c$ m^{-3}, respectively. The micronutrients Cd and Cr showed values below the quantification limit, and the Pb was 27.00 mg kg^{-1}.

For the experiment, sludge was collected from a whey processing industry, at Marechal Cândido Rondon city, western region of Paraná state, which is considered "solid waste Class II A". This sludge, which comes from the secondary decanters of an aerated lagoon that makes up the company's activated sludge system, is subject to dewatering in a centrifugal decanter (UCD 205 from GEA Westfalia) and reaches an average solids concentration of 18%.

The chemical characteristics of the solid waste from this process were determined, such characteristics can be observed and they are: N = 6.53, P = 0.98 g kg^{-1}, K = 0.78 g kg^{-1}, Ca = 1.89 g kg^{-1}, Mg = 1.29 g kg^{-1}, organic matter = 94.19 g kg^{-1}. The chemical characteristics of micronutrients are: Cu = 5.34 mg kg^{-1}, Zn = 15.03 mg kg^{-1}, Mn = 13.85 mg kg^{-1}, Pb = 8.70 mg kg^{-1}, Cr = 3.56 mg kg^{-1}, Fe = 440.00 mg kg^{-1}, and Cd presented below of the quantification limit.

The maximum permissible concentrations of Cu, Zn, Cd, Pb and Cr in the sewage sludge for agricultural use, as determined by Annex 6 of SEMA Resolution 021/09 [17] and the concentrations recommended for agricultural soil, was determined according to CONAMA Resolution N°. 420/09 [18]. The grass used in the experiment was Tifton 85 (*Cynodon* spp.), which seedlings were removed from the experimental farm of Pontifical Catholic University of Paraná, and replanted in plastic pots in April 2015, totaling 6 seedlings per pot. One month after planting to standardize height, the seedlings were cut 12 cm from the soil, then sludge was applied.

The experimental design was completely randomized (CRD), with five doses of dehydrated sludge, constituting 0, 10, 20, 30 and 40 m^3 ha^{-1} respectively, each dose with six replications, totaling 30 pots.

After 47 days of application of biosolids, to determine the dry mass, grasses were cut at 12 cm from the soil, oven dried at 60℃ for 48 hours, the leaf tissue was ground and digested with acid (nitroperchloric) mixture [15], allowing the determination of the bioaccumulated levels of P [19], K, Ca, Mg, Cu, Zn, Fe, Mn, Cd, Pb and Cr [14].

The data were tabulated and submitted to analysis of variance by the SISVAR software [20] and the regression analysis was performed when significant difference between doses were found.

3. RESULTS AND DISCUSSION

The mean squares from ANOVA for the parameters: Dry matter (DM) of Tifton 85 and for leaf contents of crude protein (CP), N, P, K, Ca, Mg, Cu , Zn, Mn, Fe, Cd, Pb and Cr at 47 days of culture, were showed in the Table 1.

Table 1 shows significant differences (P <0.01) for N and P foliar contents in Tifton 85, at 47 days of cultivation (1st cut), evidencing a high statistical difference for these parameters between the doses of biological sludge applied.

Table 1. Mean squares of analysis of variance for the parameters: dry matter (DM) and foliar leaf contents of crude protein (CP), N, P, K, Ca and Mg at 47 days

FV	MS	CP	N	P	K	Ca	Mg
Doses	1,41 ^{ns}	27,15 **	69,52 **	18,28 **	27,14 ^{ns}	0,84 ^{ns}	0,06 ^{ns}
Residue	3,38	7,49	19,18	4,97	13,04	3,00	0,13
CV (%)	37,43	12,66	12,66	25,76	14,80	12,56	10,32

*CV (%): Coefficient of variation; NS: not significant; **: significant at the 1% by Fisher's test*

Increasing doses of biological sludge did not result in increase of DM by the *Cynodon* spp. forage, however, considering that such solid residue is a problem for the industry, this result becomes interesting, since the disposal of sludge in soil not adversely affect the development of plants. However, the lack of increment in the dry matter can be explained due to the short time of cultivation, since other authors obtained elevation of DM by applying solid residues as biofertilizer only after the third cut of the forage [21].

In Table 2 it is possible to observe significant differences (P <0.01) for the leaf contents of Fe, Cd and Cr of tifton 85 (*Cynodon* spp.) at 47 days of cultivation, evidencing a high statistical difference for these parameters between the doses of biological sludge applied.

Although there was a significant difference in Cd leaf contents (Table 2), the value found for CV (%) was high. This is due to the fact that only four experimental plots showed some Cd foliar content, all other determinations being below the limit of quantification (LQ) of used analytical method. Consequently, the R^2 values for the regressions are very close to 0%.

Leaf tissue of tifton 85 showed a considerable absorption of nutrients such as nitrogen and phosphorus, however, no significant change was observed in the concentrations of heavy metals in its leaf tissue. Similar behavior was observed [22], these authors, when applying sludge from an industrial effluent treatment plant in Fenugreek (*Trigonella foenum graecum*), concluded that the consumption of this crop would not present risks to the respective consumers.

3.1 Nitrogen

Fig. 1 shows the concentration of nitrogen in the foliar tissue at doses of applied sludge, it is possible to observe the linear elevation of nitrogen according to the applied doses, indicating a strong correlation between the dose of sludge applied and the increase of absorption of nitrogen by Tifton 85, demonstrating that the application of biological sludge in the soil contributed significantly to the availability of this macronutrient in forage.

According to the [23] the nitrogen sufficiency range in the leaf tissue of *Cynodon* spp. (10 m^3 ha^{-1}) and 38.53 g kg^{-1} (40 m^3 ha^{-1}), the average nitrogen content in the present study ranged from 20 to 26 g kg^{-1} dry mass, (Fig. 1), values considerably higher than recommended.

3.2 Crude Protein

Fig. 2 shows the adjustment profile of the crude protein in relation to the different doses of biological sludge. It is possible to notice that the growth was linear, indicating satisfactory nutritional status and increment of crude protein with the addition of sludge.

In treatment with dairy wastewater in constructed flooded systems and cultivated with forage (Tifton 85 and elephant grass), it was verified that the addition of residue to soil increased the potential supply of crude protein for plants, both when considering quantity and when considering harvest time [24]. A study conducted [25], using different doses of dairy wastewater in mombasa forage, showed that the increase of doses in soil increased the content of crude protein from pasture.

[26], irrigating crop areas of *Cynodon* spp. with swine manure, obtained crude protein in forage dry mass varying from 14.21% to 16.91%, in the present study the application of biological sludge resulted in a mean variation of 20.85% to 24.08% (Fig. 2). According to [24], the minimum crude protein content required in the dry matter of forage supplied to cattle (adults and young) is around 7 to 11%, emphasizing the good results obtained in the present study.

Table 2. Mean squares of the analysis of variance for leaf contents (*Cynodon* spp.) of Cu, Zn, Mn, Fe, Cd, Pb and Cr at 47 days

FV	Cu	Zn	Mn	Fe	Cd	Pb	Cr
Doses	3,05 ^{ns}	1830,38 ^{ns}	446,72 ^{ns}	3856,30 **	2,13 **	0,00	3,13 **
Residue	4,14	15,73	203,13	975,62	0,45	0,00	0,26
CV (%)	20,92	59,59	25,63	16,25	252,49	0,00	15,93

*CV (%): Coefficient of variation; NS: not significant; **: significant at the 1% level by Fisher's test*

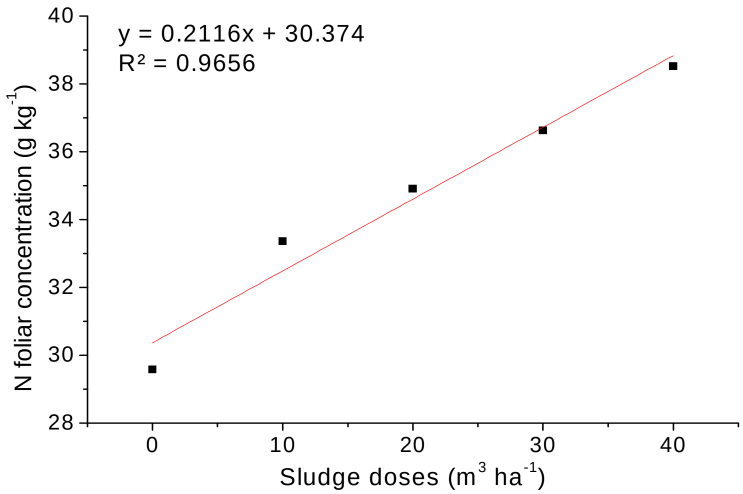

Fig. 1. Nitrogen contents in leaf tissue at different doses of applied sludge

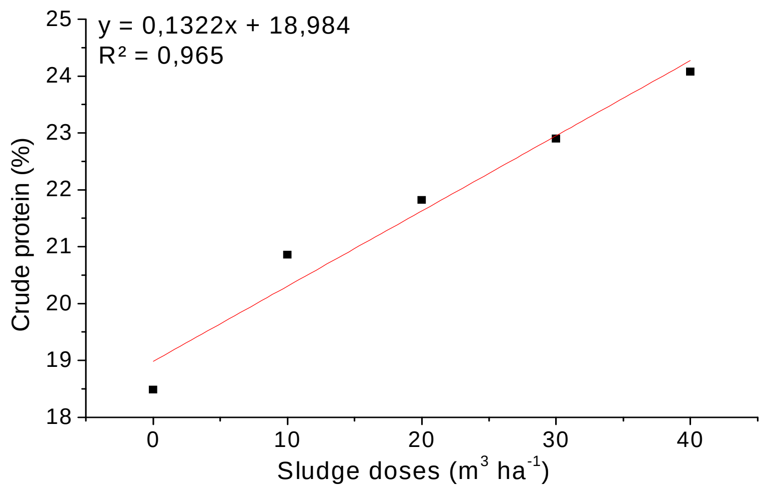

Fig. 2. Crude protein contents in leaf tissue at different sludge doses

3.3 Phosphorus

Concentrations of phosphorus in the plant tissue increased with doses (Fig. 3). A peak occurred at a dose of 30 m³ ha^{-1}, which presented the mean concentration of 1.16 g kg^{-1} of P in the forage.

The mean values of phosphorus in this experiment were higher than those found [21], which performed fertigation of tifton 85 with percolated urban solid waste and obtained 0.64 g kg^{-1} of P as the maximum concentration in the vegetable tissue, below even the lowest average value found with the application of biological sludge 0,73 kg kg^{-1}. [27] concluded that the application of biosolids in sugar cane provided the necessary amount of phosphorus for the plants development, without causing deleterious effects to soil.

The mean concentrations of phosphorus in plants varied from 0.073% to 0.116%, from a nutritional point of view, the amount of phosphorus was below 0.56%, recommended for feeding dairy cows with an average weight of 450 kg and an average yield of 25 kg per day. Thus, this solid residue contributes to increase the nutritional value of forage without causing accumulation of phosphorus, because such accumulations in bovine feed can causes the return of P through urine and stool, which can cause environmental problems [28].

3.4 Iron

Fe content in the leaf tissue showed elevation between 0 to 20 m³ ha^{-1}, in the highest doses of sludge 30 and 40 m³ ha^{-1}, the iron absorption by the plants showed a significant decrease, according to Fig. 4.

The reduction of Fe present in foliar tissue at higher doses of sludge may have occurred due to the elevation of Mn concentration in soil, when in high amounts, it inhibits Fe absorption by the plant, in this experiment Fe concentrations in leaf tissue and the amount of Mn applied in the soil correlated negatively (ρ = -0.5364). Similar situation was observed in the cultivation of tanzania grass, where high concentrations of Mn resulted in symptoms of iron deficiency by the competitive inhibition of these two micronutrients [29].

Fe absorption is influenced by cations such as K, Ca and Mg, however Cu, Zn and Mn can presumably cause iron deficiency by the competitive inhibition between these elements [30]. Studying the nutrient variability in a corn crop, these authors found a negative correlation between the Fe and Mn concentrations in aerial part of plant. Fig. 5 shows the interaction between Fe and Mn in plant tissue.

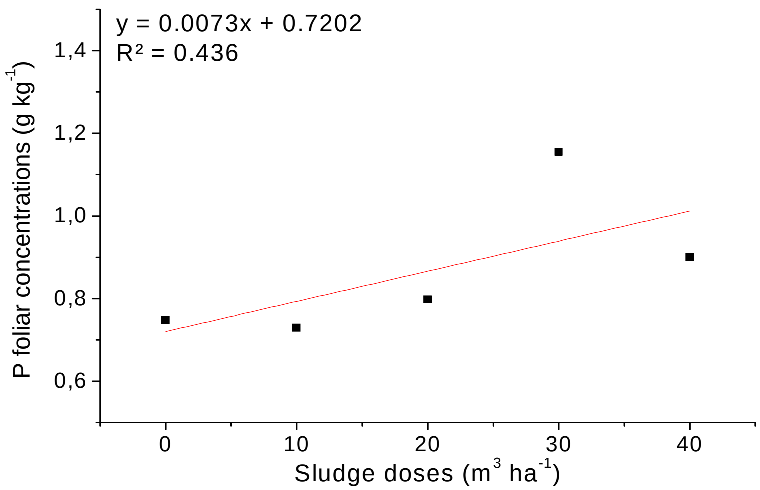

Fig. 3. Mean concentrations of phosphorus by the different doses of applied sludge

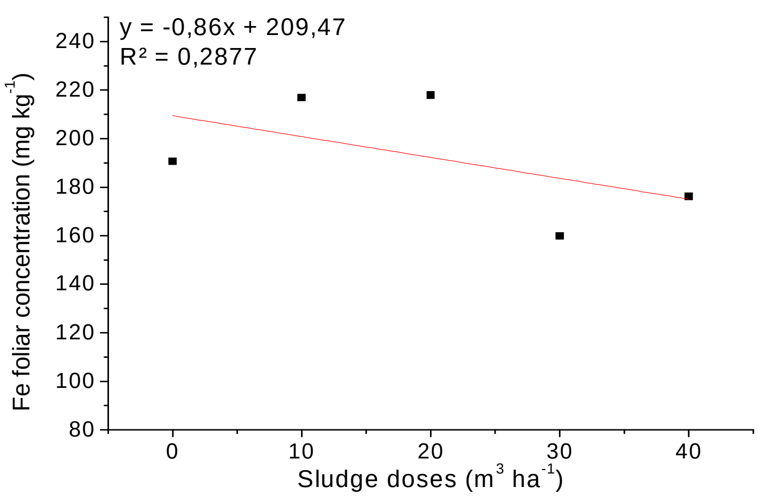

Fig. 4. Leaf content of Fe in different doses of applied sludge

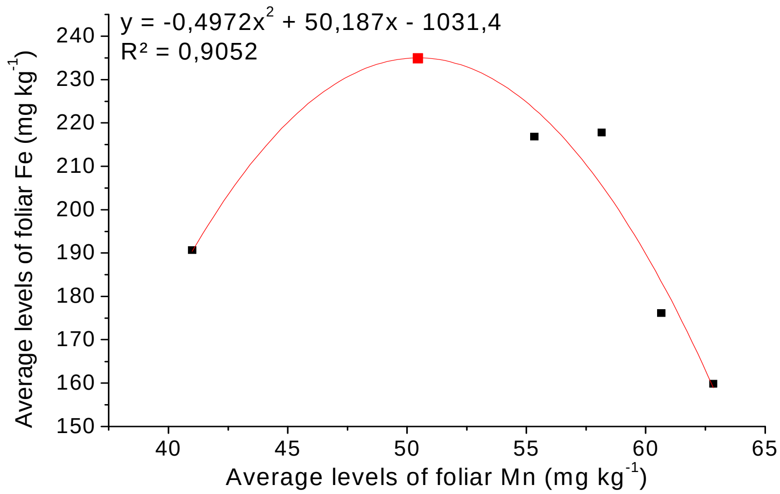

Fig. 5. Interactions between the Fe and Mn leaf contents

3.5 Chromium

With the exception of the sludge dose of 10 m³ ha^{-1}, the Cr content remained basically constant. It can be inferred from this data that the addition of greater amount of sludge to plant does not result in a higher leaf content of this heavy metal, according to Fig. 6.

Cr is absorbed with Fe and accumulated in plant roots, with low translocation for its aerial part [31], this author affirms that it is very difficult to determine a correlation between Cr concentration in soil and plant, even if normally the Cr content in plants roots increases with the increase of Cr content in soils.

In a study with corn plants, the addition of different doses of Cr showed that this was the metal that presented the highest concentration in soil and, at the same time, was not detected in leaves (<0.19 mg kg^{-1}). This can be explained by the fact that heavy metals, mainly Cr, have low absorption and translocation in plant tissues, accumulating generally in roots [32].

[33] cultivating conilon coffee plants and adding 0, 10, 20, 30 and 40% tannery sludge containing Cr to the soil, compared the average levels of chromium in the leaves, and observed that only treatment with 20% of sludge presented significant differences in relation to control, so the hypothesis presented was that even with

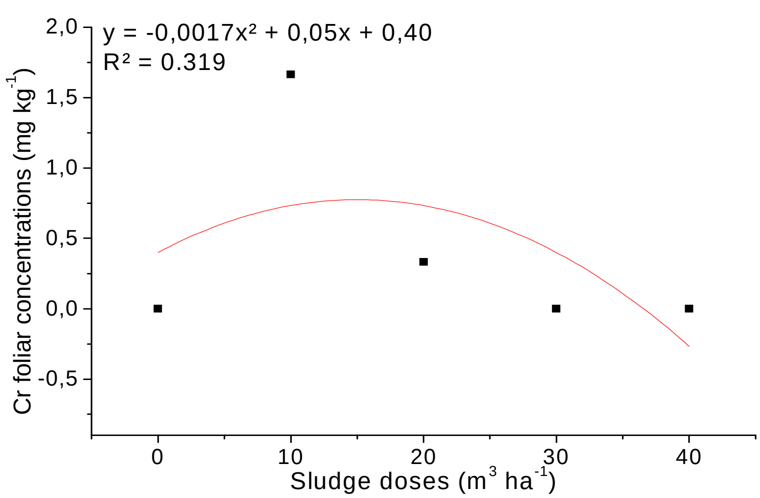

Fig. 6. Chromium content by applied sludge rates

increasing doses of sludge containing Cr, there was almost no change in Cr levels in leaves, indicating that the Cr may not be the limiting factor in the development of the leaf part of seedlings. Moreover, for the same authors, these amounts of Cr do not present toxicity to the aerial part of plants.

The toxicity of chromium in plants varies according to their oxidation state (Cr^{3+} or Cr^{6+}) and plant tolerance [34]. In their study, the author reports toxic levels of Cr in leaves, above 5.8 mg kg^{-1} for the presence of Cr^{3+}, and above 3.4 mg kg^{-1} when there is a predominance of Cr^{6+}. Confirming the statement by [35], Cr^{6+} has higher toxicity than Cr^{3+}. One of the factors that credit hexavalent chromium to this high potential for toxicity is the great ability to penetrate into cells.

It is believed, by the conditions presented in the experiment, that the presence of Cr occurred predominantly in its trivalent form, since the tests were conducted in a soil with slightly acidic pH and with organic matter in abundance, because in acid soils and with high concentrations of organic matter Cr^{6+} is easily reduced to its Cr^{3+} form [36].

4. CONCLUSION

The application of biological sludge did not have negative influence on the development of the tifton 85 (*Cynodon* spp.). The application contributed significantly to the increase of N, P and CP in forage, doses that provided concentrations of Mn higher than 50 mg kg^{-1} in the leaf tissue reduced the absorption of Fe by the plant. There was no significant absorption of metals such as Cr, Cd and Pb by the plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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