



## ROLE OF GROWTH REGULATORS AND MICROBES FOR METAL DETOXIFICATION IN PLANTS AND SOIL

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### Abstract

The mitigation effect was shown by an exogenous application of endomycorrhizas in the soils (T3) by a reduction of 27.88 and 27.14 percent compared with T0 of the proposed interval dates of total carotenoids. The mitigating effect was demonstrated by the exogenous application of endomycorrhiza to soil (T3) by decreasing anthocyanin content with 27.88% and 27.14% as opposed to T0 at the proposed interval dates. The natural factors which are responsible for the entry of heavy metals into the environment include soil erosion, mineral weathering, and volcanic eruptions. The various anthropogenic process involved in the release of toxic heavy metals in the air, water, and soil through various processes such as, tanning of leather, electroplating of metals, printing, thermometers, glass, batteries and metallurgy, dust from old paint which contains lead.

**Keywords:** Abiotic, Biotic, Cadmium, Design, Economy, Forage

### Introduction

Cadmium, lead, and arsenic are widely distributed in the environment among heavy metals (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al.*, 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. *et al.*, 2019g). However, heavy metals have a slow degradation rate due to which they can remain in the environment for a long time; which leads to the accumulation of heavy metals leads to contamination (Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i, Pathak, S., Kumar, P., P.K Mishra, M. Kumar, M. 2017j, Prakash, A., P. Kumar, 2017k., Kumar, P., Mandal, B., 2014L, Kumar, P., Mandal, B., Dwivedi P., 2014m., Kumar, P., Kumar, P.K., Singh, S. 2014n). The mobility of these heavy metals through several activities in the atmosphere such as, surface runoff and blowing winds have increased accumulation of the upper soil, contaminating air and water which has resulted in chronic illnesses of living organisms in these areas (Kumar, P. 2013o., Kumar, P., Dwivedi, P. 2015p.

Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q, Kumar, P., 2014r., Kumar, P., Dwivedi, P., Singh, P., 2012s, Mishra, P.K., Maurya, B.R., Kumar, Pp. 2012t). Road dust, roadside area and plants growing in these affected regions are subject to receive high amounts of heavy metals, from both dangerous gas emissions from motor vehicles and toxic chemicals transported (Kumar, P., Mandal, B., Dwivedi, P. 2011u. Kumar, P., Mandal, B., Dwivedi, P. 2011v, Kumar, P., Pathak, S. 2016w., Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. 2016x, Kumar, P., Harsavardhn, M. *et al.*, 2018y. Kumar, P., Yumnam, J. *et al.*, 2018z). Phytotoxic effect on plants due to heavy metals contamination results in chlorosis, inhibited photosynthesis, inhibited growth, reduced biomass and finally death of the affected plant (Kumar, P., Pandey, A.K., *et al.*, 2018aa, Kumar, P., Kumar, S. *et al.*, 2018bb, Kumar, P., Krishna, V., *et al.*, 2018cc). So, it is important to reduce the metal uptake by plants and resist the entry of metals into the food chain

which slowly reaching the highest trophic level (Singh *et al.* 2020a., Singh *et al.*, 2020b., Sood, *et al.*, 2020., Bhadrecha *et al.* 2020, Singh *et al.*, 2020c, Sharma *et al.*, 2020, Singh *et al.*, 2020d, Bhati *et al.*, 2020, Singh *et al.*, 2019, Sharma *et al.*, 2019). Cadmium one of the most toxic heavy metals having an upper limit is 14.157 µg/g (Singh *et al.* 2020a., Singh *et al.*, 2020b., Sood, *et al.*, 2020., Bhadrecha *et al.* 2020, Singh *et al.*, 2020c, Sharma *et al.*, 2020, Singh *et al.*, 2020d, Bhati *et al.*, 2020, Singh *et al.*, 2019, Sharma *et al.*, 2019). Effects of Cd, according to Sharmila *et al.* 2017, when mustard exposed to Cd<sup>2+</sup> affects the growth of the plant and reduces the activity of photosystem II with a rise in the level of proline. Affect the oxidative phosphorylation in mitochondria and water uptake (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al.*, 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. *et al.*, 2019g, Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i). The linear increase in the amount and production of MDA and H<sub>2</sub>O<sub>2</sub> during stress in roots of chickpea; inhibits the plant growth by stimulating ROS [P. Kumar 2014r]; affects the leaves, shoot, Significant reduction in the amount of nitrogen, phosphorus and chlorophyll were observed with an increase in the concentration of Cadmium; affects the translocation and storage of sugar in sweet sorghum (Pathak, S., Kumar, P., P.K Mishra, M. Kumar, M. 2017j, Prakash, A., P. Kumar, 2017k., Kumar, P., Mandal, B., 2014L, Kumar, P., Mandal, B., Dwivedi P., 2014m., Kumar, P., Kumar, P.K., Singh, S. 2014n, Kumar, P. 2013o., Kumar, P., Dwivedi, P. 2015p, Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q); reduces the internodal space and internodes number in maize. Lead (Pb) is one of the non – essential trace elements that mainly accumulate due to anthropogenic activities in agricultural soils. The upper limits of leads are 61.87 µg/g (Kumar *et al.*, 2018i). The increased levels of Pb in the soil increase the concentration of Pb in plants growing in these soils and ultimately increases the risk of Pb toxicity in food crops. Lead toxicity induces the effects chlorophyll, affects concentration and catabolism of IAA, stimulates ROS

production and also POD activity, reduced total nitrogen and total phosphorus in the plant reduction in germination (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al.*, 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. *et al.*, 2019g, Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i). Also, the reduction in the relative water content (RWC) and net photosynthetic rate (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al.*, 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. *et al.*, 2019g, Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i).

### Materials and Methods

This was the pot for the experiment with a 30 cm diameter and a 25 cm height and 10 kg of soil each with a small hole underneath it. Under the work plan, targeted pots with Endomycorrhiza have been inoculated. The exogenous use of cadmium (100 ppm) by Cadmium sulfate and Lead (100 ppm) by Lead chloride on the plant creates heavy metal stresses. Fifteen days interval application with Putrescine (1ppm) and Salicylic Acid (1ppm). Two phases such as 60 DAS and 90 DAS were measured in the respective pots. (Table 1).

**Table 1 :** Name of the Treatments and symbol used respectively.

Name of Treatments	Symbol Used For Respective Treatments
Control	T-0
Cadmium(100 ppm)	T-1
Lead(100 ppm)	T-2
Cadmium + Mycorrhiza	T-3
Lead + Mycorrhiza	T-4
Cadmium + Putrescine	T-5
Lead + Putrescine	T-6
Cadmium + Salicylic Acid	T-7
Lead + Salicylic Acid	T-8

### Design and Layout of Experiment

In a completely randomized (CRD) design, the experiment was developed. Eight treatments were available, including control. Three times every treatment has been replicated.

### Observation Recorded

The observations were recorded two stages such as 60 DAS, and 90 DAS. The recorded observations of biochemical parameters and the standard procedure adopted during the study are given below:

### Anthocyanin content (mg g<sup>-1</sup> fresh weight)

The method described by Swain and Hills (1959) to measure anthocyanin in the plant sample. The alcohol extract of the sample is treated with HCl in aqueous methanol followed by anthocyanin reagent. The colour intensity is measured calorimetrically at 525nm. Grind a known weight of fresh plant material in alcohol. Filter or centrifuge and collect the extract. Pipette 1 ml of the alcohol extract into the test tube and add 3ml of HCl in aqueous methanol. Add 1ml of anthocyanin reagents to the sample. Prepare the blank in

the same manner by adding 1ml of methanol-HCl instead of anthocyanin reagent. After 15 min of incubation in the dark, measure the absorbance at 525nm against the blank. Calculate the amount of anthocyanin present in the sample from a standard curve prepared with cyanin hydrochloride. One gram of cyanin hydrochloride was dissolved in 100ml of methanol-HCl solvent. From this stock solution, a different concentration of cyanin hydrochloride solution was prepared by taking 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the stock in a separate test tube. The final volume of these test tubes was made by 1 ml by adding distilled water. The standard curve was prepared by plotting the absorbance value at 525nm on the y-axis, against the concentration of cyanin hydrochloride in solution on the x-axis.

### Total Carotenoids content

The method described by Jensen A. (1978) for the determination of total Carotenoids in the plant sample was followed. The total Carotenoids are extracted and portioned in an organic solvent (acetone or methanol) based on their solubility. Carotenoids that are bound as esters are hydrolyzed using aqueous 60% KOH. The amount of the Carotenoids present in the sample is estimated calorimetrically at 450nm using  $\beta$ -carotene as a standard. Cut the fresh plant material and grind a known amount (2g) in a mortar with 20 ml of either distilled acetone or methanol. Filter on a Buchner funnel through Whatman No. 42 filter paper. Repeat the extraction until the tissue is free from pigments. Pool the filtrate thrice with an equal volume of peroxide-free ether using a separatory funnel. Evaporate the combined ether layer (which contains the Carotenoids) under reduced pressure at 35°C in a rotary evaporator or a hot water bath. Dissolve the residue in a minimum quantity of ethanol. Add 0% aqueous KOH at the rate of 1ml for every 10ml, of the ethanol extract to saponify. Keep the mixture in dark and leave it overnight at room temperature. Add an equal volume of water and partition with ether. Evaporate the combined ether layer as before and dissolve the residue in a minimum volume of ethanol. Measure the absorbance of this solution at 450nm and calculate the Carotenoids content in the sample using a calibration curve prepared against a high purity of  $\beta$ -carotene. One gram of  $\beta$ -carotene was dissolved in 100 ml of acetone or methanol solvent. From this stock solution, different concentrations of  $\beta$ -carotene solution were prepared by taking 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the stock in separate test tubes. The final volume of these test tubes was made by 1 ml by adding distilled water. The standard curve was prepared by plotting the absorbance value at 450 nm on the y-axis, against the concentration of  $\beta$ -carotene on the x-axis.

## Results and Discussion

### Anthocyanin Content (mg g<sup>-1</sup> fresh weight)

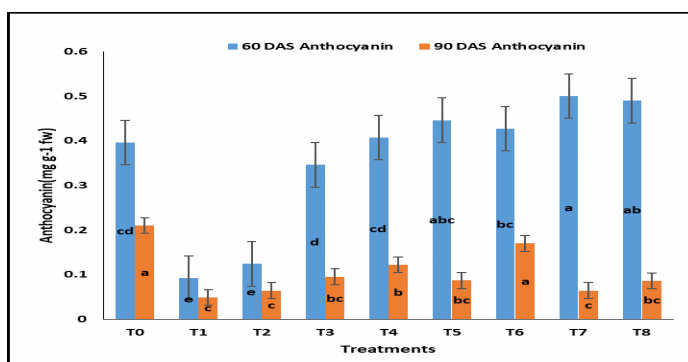
In chickpea variety, GPF-2 cadmium and lead stress were examined to evaluate its effect on the content of polyamine (putrescine), mycorrhiza, salicylic acid, and their combination. 60 and 90 days after sowing (DAS) (Table 2, Fig. a) data were registered. It is clear that with cadmium metal stress (T1) exposed at dates of 60 and 90 DAS interval the average anthocyanin content was significantly reduced with 30.17 and 27.96 percent in comparison to control (T0), respectively. Similarly, the anthocyanin level was reduced considerably with 38.63% and 46.65%, compared with control (T0) at the proposed interval, when exposed to an elevated dose of plant lead (T2). The effect of mitigation by

reducing the level of anthocyanin to 27.88 percent and 27.14 percent compared to T<sub>0</sub>, on the proposed dates of the interval, exogenous application of endomycorrhizal to soil (T<sub>3</sub>). Similarly, the anthocyanin content was significantly reduced by 25.52% and 26.87% on the proposed interval date when the treatment T<sub>4</sub> was compared to T<sub>0</sub>. The exogenous use of putrescine (T<sub>5</sub>) showed anthocyanin concentration mitigation of 27.15% and 16.79% on the proposed interval date, compared to T<sub>0</sub>. When treated with a higher dose of putrescine (T<sub>6</sub>), the average anthocyanin level was significantly reduced by 24.35% and 12.13% compared to T<sub>0</sub>. Similarly, the anthocyanin level decreased significantly when T<sub>7</sub> was compared with T<sub>0</sub> at 17.52% and 10.13% on the proposed interval date. The average amount of anthocyanin in treatment with a higher dose of salicylic acid was reduced considerably compared to T<sub>8</sub> with 22.97% and 6.11%. Salicylic acid showed the best effect on cadmium mitigation and reduced the amount of anthocyanine on the proposed interval date. Imtiaz *et al.* (2016) experimented to elucidate the effect of vanadium (V) on the following genotypes of chickpeas: C-44 (tolerant) and Balkasar (sensitive) in the field of photosynthetic pigments, membrane damage, antioxidant enzymes, protein and deoxyribonucleic acid (DNA). These parameters were significantly affected by V levels, by DNA damage induced in Balkasar only at 60 and 120 mg V<sup>-1</sup>, while photosynthetic pigments and protein decreased in Balkasar from 15 to 120 mg V L<sup>-1</sup>, with damage to the membrane as well. Photosynthetic pigments and protein production were found to decrease between 15 and 120 mg V L<sup>-1</sup> and membrane damage, whereas DNA damage at V levels at-44 was not observed.

**Table 2 :** Anthocyanin content (mg ml<sup>-1</sup>) of chickpea during *Rabi*

Treatments	Anthocyanin (60 DAS)	Anthocyanin (90 DAS)
T <sub>0</sub>	0.396 <sup>cd</sup> ± 0.015	0.210 <sup>a</sup> ± 0.010
T <sub>1</sub>	0.092 <sup>e</sup> ± 0.014	0.049 <sup>f</sup> ± 0.023
T <sub>2</sub>	0.124 <sup>e</sup> ± 0.008	0.064 <sup>e</sup> ± 0.018
T <sub>3</sub>	0.346 <sup>d</sup> ± 0.023	0.095 <sup>bc</sup> ± 0.015
T <sub>4</sub>	0.407 <sup>cd</sup> ± 0.055	0.122 <sup>b</sup> ± 0.010
T <sub>5</sub>	0.446 <sup>abc</sup> ± 0.006	0.087 <sup>bc</sup> ± 0.018
T <sub>6</sub>	0.427 <sup>bc</sup> ± 0.015	0.170 <sup>a</sup> ± 0.010
T <sub>7</sub>	0.500 <sup>a</sup> ± 0.012	0.064 <sup>e</sup> ± 0.011
T <sub>8</sub>	0.490 <sup>ab</sup> ± 0.010	0.086 <sup>bc</sup> ± 0.018

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T<sub>0</sub>-Control; T<sub>1</sub>-Cadmium (100ppm); T<sub>2</sub>-Lead (100ppm); T<sub>3</sub>-Cadmium + mycorrhiza; T<sub>4</sub>- Lead + Mycorrhiza; T<sub>5</sub>- Cadmium + Salicylic acid (1 ppm); T<sub>6</sub>-Lead + Salicylic acid (1 ppm); T<sub>7</sub>-Cadmium + Putrescine (1 ppm); T<sub>8</sub>-Lead + Putrescine (1 ppm)



**Fig. a :** Anthocyanin content (mg ml<sup>-1</sup>) of chickpea during *Rabi*

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T<sub>0</sub>-Control; T<sub>1</sub>-Cadmium (100ppm); T<sub>2</sub>- Lead (100ppm); T<sub>3</sub>-Cadmium + mycorrhiza; T<sub>4</sub>- Lead + Mycorrhiza; T<sub>5</sub>- Cadmium + Salicylic acid (1 ppm); T<sub>6</sub>-Lead + Salicylic acid (1 ppm); T<sub>7</sub>-Cadmium + Putrescine (1 ppm); T<sub>8</sub>-Lead + Putrescine (1 ppm)

### Total Carotenoids Content (mg ml<sup>-1</sup>)

In chickpea GPF-2 cadmium and lead stress, effects were investigated of polyamine (putrescine), mycorrhizas, salicylic acid, and their combination on the overall carotenoid level. Data were recorded at 60 and 90 days after sowing (DAS) (Table 3, Fig. b). It is obvious that, when cadmium metal stresses (T<sub>1</sub>) were exposed, the average total carotenoid content was significantly reduced by 30.17% and 27.96% compared to control (T<sub>0</sub>) at 60 and 90 DAS intervals. Similarly, the total content of plants with a higher dose of lead (T<sub>2</sub>) was significantly decreased with the proposed interval of carotenoids, at 38.63% and 46.65% in comparison to control (T<sub>0</sub>). The mitigation effect of the exogenous application of endomycorrhizal (T<sub>3</sub>) in the soil was shown through a decrease of 27.88% and 27.14% over the T<sub>0</sub> on the proposed interval dates. Likewise, the combined carotenoid content of treatment T<sub>4</sub> was significantly reduced compared to T<sub>0</sub> at the proposed interval dates, at 25.52 and 26.87 percent. The exogenous application of putrescine (T<sub>5</sub>) showed mitigation of total carotenoid content at the proposed interval date of 27.15% and 16.79% compared to T<sub>0</sub>. Compared to T<sub>0</sub> in the case of higher doses of putrescine, the mean overall content of carotenoids decreased significantly by 24.35% and 12.13% (T<sub>6</sub>). Similarly, the total carotenoid content was lower compared to T<sub>0</sub> in treatment T<sub>7</sub> at the proposed interval of 17.52 percent and 10.13 percent. In comparison to T<sub>8</sub> with 22.97 percent and 6.11 percent in the case of higher dose salicylic acid (T<sub>0</sub>), the average total carotenoid content was significantly decreased. The salicylic acid showed the best mitigation effect against the cadmium and lead by decreasing the total carotenoids content on the proposed date of interval. Yi *et al.* (2018) have reported the positive effects on crops under salt stress of exogenous spermidine (Spd, some polyamine), however little information about Spd's effects on combined treatment for enriching CO<sub>2</sub> and the effects of iso-osmotic salt stress is available. In tomatoes (*Solanum Lycopersicum* L.) we have investigated the effects of exogenous Spd (0.25 mM) on plant growth (CO<sub>2</sub> enrichment (800 ppm) and isoosmotic salt stress (150 mmol / L NaCl and 100 mmol / L Ca (NO<sub>3</sub>)<sub>2</sub>).

**Table 3 :** Total carotenoids content (mg ml<sup>-1</sup>) of chickpea during *Rabi*.

Treatments	Carotenoids (60 DAS)	Carotenoids (90 DAS)
T <sub>0</sub>	6.820 <sup>a</sup> ± 0.043	3.680 <sup>a</sup> ± 0.003
T <sub>1</sub>	4.762 <sup>c</sup> ± 0.153	2.651 <sup>d</sup> ± 0.008
T <sub>2</sub>	4.185 <sup>f</sup> ± 0.187	1.963 <sup>e</sup> ± 0.331
T <sub>3</sub>	4.918 <sup>de</sup> ± 0.047	2.681 <sup>d</sup> ± 0.006
T <sub>4</sub>	5.079 <sup>cde</sup> ± 0.037	2.691 <sup>d</sup> ± 0.008
T <sub>5</sub>	4.968 <sup>cde</sup> ± 0.040	3.062 <sup>c</sup> ± 0.003
T <sub>6</sub>	5.159 <sup>cd</sup> ± 0.030	3.233 <sup>bc</sup> ± 0.008
T <sub>7</sub>	5.625 <sup>b</sup> ± 0.158	3.307 <sup>bc</sup> ± 0.004
T <sub>8</sub>	5.253 <sup>c</sup> ± 0.009	3.455 <sup>ab</sup> ± 0.007

where, DAS: Days after sowing, Data are in the form of Mean $\pm$ SEM at  $p>0.05$ , T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid (1 ppm); T6-Lead + Salicylic acid (1 ppm); T7-Cadmium + Putrescine (1 ppm); T8-Lead + Putrescine (1 ppm)

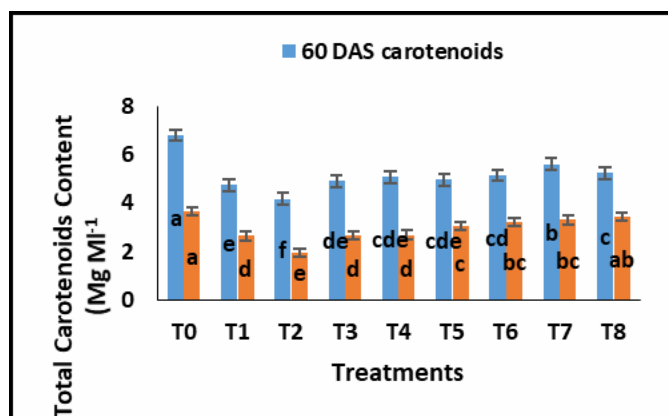


Fig. 1b : Total Carotenoids Content (mg ml<sup>-1</sup>) of Chickpea during Rabi

where, DAS: Days after sowing, Data are in the form of Mean $\pm$ SEM at  $p>0.05$ , T0-Control; T1-Cadmium (100ppm); T2- Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid (1 ppm); T6-Lead + Salicylic acid (1 ppm); T7-Cadmium + Putrescine (1 ppm); T8-Lead + Putrescine (1 ppm)

### Conclusion

Growth regulators and fungi significantly alleviate the toxicity of cadmium to chickpeas by increasing the defensive role of carotenoids and anthocyanin pigments in chickpea. Polyamines are present in almost all living organisms and also in the plant). Polyamines are helpful in growth and development, also respond during abiotic or biotic stress, the Pas are present in trace amounts like putrescine but in mammal's spermidine and spermine are present. The symbiosis of plant roots with fungi occurs in various forms known as mycorrhiza. Arbuscular mycorrhizal fungi (AMFs) are major soil microorganisms that are key to enabling plant nutrient uptake, particularly in low-input farming, vegetation, and rhizoremediation processes, in various agroecosystems. Salicylic acid (SA) a compound which has been used to reduces the heavy metals toxicity in plants, which helps in the regulation of plant growth.

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### Author Contributions

The study was designed by P.K. and M.N, the biochemical protocolizations were established, experiments were carried out and the data analyzed and interpreted were collected. The paper has been written by P.K.

### Conflict of Interest Statement

The authors declare no conflict of interest.

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