Electro-enhanced phytoremediation system on the removal of trace metal concentration from contaminated water

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ABSTRACT
The combination of electro-enhanced and hydroponic phytoremediation hereinafter referred to as electro-enhanced phytoremediation (EP) system, has been employed for rapid removal of trace metal concentration of lead (II) from contaminated water using Kentucky bluegrass (Poa pratensis L.) as accumulator plant. In this study, for rapid assessment the effectiveness of two-dimensional (2D) electrode configuration in electro-enhanced system was evaluated by agar media for 48h period of time. Furthermore, these configurations were applied to enhance the EP system for 9d period of time. Also, a common agrochemical-urea as chaotropic agent to facilitate the healthy growth of plant in contaminated water was evaluated. The results showed that the accumulation of lead (II) concentration was higher in the plant roots (i.e. high bioaccumulation coefficient (BC) value) than in aerial parts of plant (i.e. low translocation factor (TF) value). Also, the accumulation of lead (II) concentration in plant was higher under the treated urea of EP system. The chlorophyll content, biomass accumulation productivity, and water content (i.e. dry weight-fresh weight (DW/FW) ratio) of plant either under the treated urea or untreated urea with high accumulation of lead (II) concentration revealed that the Kentucky bluegrass has able to hold out the plant stress.

1. Introduction
Heavy metal pollution in soil and groundwater has become an important environmental issue in the world. Abandoned mines, metal refiners, and steel-making industries are major sources for metal contamination [1]. Lead (Pb) is a typical and dangerous water and soil contaminant because it has high potential toxicity and easy to spread in multiple environmental media such as soil, dust, water food and air [2]. Additionally, lead speciation tends to associate with other mineral phases, e.g. Fe and Mn oxides, sulphides, carbonates, phosphate, hydroxide, as well as it can be retained by organic matters, oxide and clay minerals [3]. A serious effort to treat polluted soil and water has led to the development several techniques, including bioremediation, chemical washing, soil vapor extraction, water flushing, adsorption, pump-and-treat methods, etc. [4, 5]. Among listed remediation technologies, phytoremediation is one of suitable cost-effective biological remediation for contaminated soil without affecting soil fertility [6].

Phytoremediation is a green technology to remove, degrade, or accomplished hazardous contaminants in the soil or groundwater, which involves the plant and its processes [7]. Unfortunately, these remediation techniques have some drawbacks. For example, (i) requires a long treatment time, (ii) clean-up depth depend on the length of plant roots, (iii) can be applied in low or moderate concentration of contaminant, and (iv) the removal of pollutant is controlled by growth and biological cycles [8, 9, 10]. Thus, bioavailability of plant adsorption the contaminant from soil is very low where the phytoremediation technique cannot be employed properly. To overcome these limitations, coupling of phytoremediation with electro-enhanced (EP) system in soil remediation has been first reported [11] and this technique has recently proposed to improve the plant uptake of lead (II) from contaminated soil [9, 12, 13, 14]. The method has overcome the drawbacks of phytoremediation such as short period of time on remediation of contaminated site [14], and the techniques can be applied to extent the limitation of plant root in depth contaminated plume [15].
Recently, several applications of EP system in the laboratory scale have been reported on the removal of heavy metal (i.e. Cd, As, Cu, Pb) from contaminated water, and up-take by *Lactuca sativa*, *Lemna minor*, *Eichornia crassipes*, and *Pistia stratiotes* [16, 17, 18, 19]. Additionally, this method had been evaluated under the actual conditions to predict the viability of EP system in the future on the remediation of industrial effluents [20, 21, 22]. Mostly, the method was performed in the hydroponic system to eliminate the effect of extrinsic on the phytoremediation of soil variables include the temperature, precipitation, insect attack and geological background [14]. Some advantages on the application of EP system in the contaminated water are as follow: (i) hydroponic system can be applied as flexible and faster assessment on the selection of appropriate plants, and (ii) this method is easy to use on evaluation the better material for the electrode configuration (i.e. 1D or 2D) in the electrokinetic remediation [15, 18, 19]. However, the EP system has a limit applicability on the water remediation which is earlier phytotoxicity symptom in the of plant growth, so that the plant quickly experience to death because of rapid adsorption of heavy metal [18, 19]. Fortunately, these problems recently are addressed by the air purging to increase dissolved oxygen [23, 24]. or amended with urea to support the healthy grow of plant in the contaminated water [15].

Grasses are prospective candidates for phytoremediation purposes due to hardy in nature, rapid growth, hairy and deep-root system, high biomass-producing, fast growth, tolerant to toxic effects of metals and contaminants, adaptations to infertile soils, and successive shoot regrowth after harvest, among the reason [25, 26, 27]. Although most grasses have desirable characteristics for trace element phytoremediation [28], but their ability is depending on the trace element bioavailability, the mechanisms involved in the uptake, transport, accumulation, toxicity, tolerance to each trace element, and the cultivation system [6]. To the best of our knowledge, Kentucky bluegrass (*Poa pratensis* L.) has never been tried in the hydroponic of EP system on the remediation of contaminated water. The plant can grow fast to produce adequate biomass; besides, it has been known for its high macronutrient demand [29]. Moreover, the plant has high tolerant against the heavy metals [15, 29, 30]. Therefore, this plant could have a high potential to be used in the EP system.

Herein, we report the rapid assessment on the removal of lead (II) with the EP system from contaminated water and uptake by Kentucky bluegrass (*Poa pratensis* L.) as the accumulator plant. The enhancement of hydroponic phytoremediation with EP system was performed and evaluated by: (i) the effectiveness of 2D electrode configuration, (ii) the survival indicator on the level of lead (II) tolerance in the plant; (iii) the efficiency of root-to-shoot translocation and accumulation of lead (II) concentration in the plant; (iv) the implication of urea as the chaotropic agent for the last longer of plant growth.
2. Materials and methods

2.1. Materials and hydroponic set-up

All chemicals were purchased in pure grade from Wako Pure Chemicals, Co (Tokyo, Japan) unless stated otherwise. Kentucky bluegrass (Poa pratensis L) was purchased from G-GAIA Co., Ltd (Sapporo, Hokkaido, Japan) in the dimension of 200 (length, L) x 30 (width, W) x 2 (height, H) cm. Plants were prepared according to elsewhere reported study before used in the experiment [15]. Briefly, plant was sliced in the dimension of 30 (L) x 20 (W) cm before planted in the plastic trays which containing a half-strength of Hoagland solution. All plants were accommodated in the acclimated rack for three weeks of acclimatization period. The acclimated rack was constructed by the dimension of 180 (L) x 60 (W) x 180 (H) cm which was installed by fluorescent tube (NEC Biolux FL 4 SBR) and timer to provide 16/8 h light/dark cycle as the light source for growth system. In this regard, the acclimated rack kept the temperature at 24–28 °C in day light and 18–20 °C in dark night, and the humidity at 40–45%. The half-strength of Hoagland solution provided in study was prepared based on the modified procedure according to elsewhere reported study by mixing KNO3 (0.253 g), KH2PO4 (0.0676 g), MgCl2.6H2O (0.20 g), and Ca(NO3)2.4H2O (0.59 g) in 1 L deionized water [31, 32]. All mixed solutions were adjusted by a few drops of 1 M NaOH until pH 6.75. In this regard, there was no metal hydroxide precipitation observed in the hydroponic solution.

2.2. Evaluation of 2D electrode configuration

The aim of this study was to evaluate a designed 2D electrode configuration that effectively can be used to enhance the EP system as shown in Figure 1. A square polypropylene tray with the dimension of 290 (L) x 160 (W) x 95 (H) mm was used for the hydroponic growth. Four bundles of graphite anode (e.g. 7 rods in each bundle, 130 mm long, Ø 2 mm) and combined stainless steel rod SUS316 (200 (L) x 100 (W) mm, 3 mm thickness) and stainless steel net wire (200 (L) x 100 (W) mm, 20 mesh) were assembled together as cathode electrode. The electric currents were recorded during the testing period by mid logger (GL200A, Graphitec, Tokyo, Japan).

To evaluate the effectiveness of 2D electrode configuration, an agar matrix was prepared to replace the aquatic media on rapid assessment of EP system for 48h of testing period. The contaminated agar was prepared by pouring 100 g agar in 3.8 L mixture volume of 0.0025 M KNO3 and 150 mg/L Pb(NO3)2 solution. Further results from this step were applied on the remediation of lead (II) contaminated water in EP system at 50 mA of constant current for 9d of testing period.

2.3. Hydroponic experiment in phytoremediation and EAPR system

Two differences of treatment were evaluated in the hydroponic experiment. First, the level of initial lead (II) concentration was 300 and 500 mg/L as Pb(NO3)2 salts. Plant was grown by mixed solution of constant current for 9d of testing period.

"In this regard, there was no metal hydroxide precipitation observed in the hydroponic solution."

For lead (II) measurement, 25 mL solution was sampled from tray daily, and then a few drops of 5 M HCl were added before stored in glass vials capped with a paraffin. The method was optimized based on the proper atomic lines for Pb (283.306 nm) by flame-AAS (Hitachi A-2000, Japan). The pH of solution was checked during the period of growth. For lead (II) measurement, 25 mL solution was sampled from tray daily, and then a few drops of 5 M HCl were added before stored in glass vials capped with a paraffin. The method was optimized based on the proper atomic lines for Pb (283.306 nm) by flame-AAS (Hitachi A-2000, Japan).

2.4. Heavy metal analysis in plant

Directly after the experiment completed, plants were picked, weighed, and then distinguished between roots and shoots part. Root was then cleaned by tap water, whereas all roots and shoots part were sliced to obtain the small size, and then continued for 2d drying at 80 °C, subsequently grounded to obtain a fine dried sample powder. For lead (II) measurement, a sample of 200 mg powder was digested overnight by mixing with 20 mL concentrated HNO3 and HClO4 (10:5 v/v). The solution was then adjusted by deionized water until 50 mL. Flame-atomic absorption spectrophotometer (Hitachi A-2000, Japan) was employed to measure the lead (II) concentration at wavelength of 283.306 nm. The concentrations of element in this study were reported in a dry matter basis. To evaluate the translocation of metal ions in shoot part, the translocation factor (TF) was calculated by the concentration ratio of metal in the plant shoots and the roots [35]. Additionally, the bio-accumulation coefficient (BC) was also calculated by the evaluation of lead (II) concentration adsorbed from the contaminated water to the root part [30].

2.5. Plant growth and physiological parameters

Chlorophyll concentration of plant was determined according to elsewhere publication [36]. Briefly, plant leaves were sliced by 0.5 cm, and then 200 mg sample was then incubated in acetone for 24 h at 4 °C in the dark room. Finally, the chlorophyll extract was measured by spectrophotometer UV-Vis (JASCO J5460, Japan) at wavelength of 645 and 663 nm. The concentration of chlorophyll (mg/ml) was calculated by Eqs. (1), (2), and (3).

\[ \text{[Chl a]} = \frac{12.7 \times \text{Absorbance } \lambda_{663} - 2.69 \times \text{Absorbance } \lambda_{645}}{1} \]  
\[ \text{[Chl b]} = \frac{22.9 \times \text{Absorbance } \lambda_{645} - 4.68 \times \text{Absorbance } \lambda_{663}}{1} \]  
\[ \text{[Total Chl]} = \frac{8.02 \times \text{Absorbance } \lambda_{663} + 20.2 \times \text{Absorbance } \lambda_{645}}{1} \]

3. Result and discussion

3.1. Evaluation of 2D electrode configuration

Figure 1 shows a schematic diagram of 2D electrode configuration in the EP system. In this system, the anodes were installed vertically in the four corners of tray which arranged on the perimeter to maximize the acidic condition generated by anode, while to minimize the alkaline condition from cathode that placed in the center. These electrodes configuration increases linearly the electrical field strength of current density from anode toward the cathode [37]. Generally, the acid front in the anode (Eq. 4) can enhance the dissolution of lead (II) ions, causing the ions to move freely in the agar media forward near to cathode electrode where they can be removed by naturally adsorption of plant [15]. However, alkaline front in the cathode (Eq. 5) has significantly declined the mobility of the lead (II) ions because of the metal hydroxide precipitation, thereby immobilizing the metals before they can reach the cathode electrode. However, the mobility of H⁺ ions was faster and continuously produced in the solution than OH⁻ ions [38]. Therefore, the pH of acid solution more developed in the agar media.

Anode: \( 2\text{H}_2\text{O}_{(l)} \rightarrow \text{O}_2_{(g)} + 4\text{H}^+_{(aq)} + 4\text{e}^-\)  
Cathode: \( 2\text{H}_2\text{O}_{(l)} + 2\text{e}^- \rightarrow \text{H}_2_{(g)} + 2\text{OH}^-_{(aq)} \)
Figure 2. Lead (II) distribution in the agar media after 48h of constant current 50 mA EP experiment. Agar media was prepared by mixture of 100 g agar in 3.8 L solution of 150 mg/L Pb(NO₃)₂ and 0.0025 M KNO₃ as a background electrolyte ($n = 3$).

Figure 3. Effect of initial lead (II) concentration (Figure 3a, i.e. 300 and 500 mg/L of lead (II) concentration, represent as EP 300 or 500 and Phyto 300 or 500) and two differences of urea concentration (Figure 3b, i.e. 0.1 and 0.01 % of urea concentration, represent as EP 0.1% and Phyto 0.1 or 0.01% in 500 mg/L of lead (II) concentration) on the pH profiles of solution, respectively.
Figure 2 shows the transportation pattern of lead (II) ion from bottom tray at the anode position toward the cathode in the end of experiment. Highly accumulation of lead (II) concentration occurred in the middle part of agar media that figured out by the V-shape toward the cathode electrode. This lead (II) concentration was as much as 85.13–238.13 mg/L from the bottom to the upper part of horizontal level in the agar media. Similar results have also confirmed by elsewhere publication [15].

Additionally, the electroosmotic phenomenon occurred in the direction from anode to cathode would improve the upward counter gravitational movement of lead (II) ions in the water. Therefore, these results have confirmed that the 2D electrode configuration in this study could be applied on the removal the lead (II) ions from depth contaminated plume toward the rhizosphere area and then naturally adsorption by plant roots.

3.2. pH profiles in the hydroponic phytoremediation and EP system

The pH of solution was monitored for a certain of testing period during the operation of EP system and phytoremediation as shown in Figure 3a. Following initial pH of solution slightly decreased (i.e. from 6.75 to 5.04), the pH of solution in the treated plant with phyto 300 and phyto 500 did not significantly change over the time. Thus, there was not potential effect in the pH of solution on presence of lead (II). However,
the pH of solution of treated plant with EP 500 system was slowly decrease over the time and afterward had a constant pH around 3.45–3.50. Contrary results showed in the pH of solution of treated plant with EP 300 system which significantly increased over the time and afterward had a constant pH around 6.65–6.75. These phenomenon were triggered by the hydrolysis of lead (II) ion in the water which contained an alkaline salt from prepared Hoagland solution [15].

The pH of solution with urea-treated trays (i.e. 0.01% or 0.1%) increased significantly over time from pH 6.75 to 7 for 5d of testing period and thereafter remaining constant at pH 8 as shown in Figure 3b. These significant increases pH may presumably induced by the formation of ammonia gas from the hydrolysis of urea since the pH tends to be alkaline compared with the initial pH and remaining unchanged [34]. Furthermore, the increase of urea concentration did not change the pH of solution. Additionally, the plant could grow well either in the alkaline or acid conditions [39].

### 3.3. Lead concentration in the plants under hydroponic phytoremediation and EP system

Figure 4 shows the concentration of lead (II) in the roots and shoots part of plant which grown in the hydroponic contaminated water. Out of eight tested experiments, the highest level of lead (II) concentration was in roots part for each treatment. Despite the high level of lead (II) concentration in the roots part as shown in high BC value (6.00–24.01), the ability of plant to translocate the lead (II) concentration from roots to shoots part were lower in the untreated-urea (TF = 0.06 to 0.16) than the treated-urea (TF = 0.04 to 0.61), revealing that the lead (II) tends to be accumulated in the plant roots.

Figure 4a shows the removal ability of lead (II) by phytoremediation was higher than EP system even though the treatment was in the same concentration of lead (II) (i.e. 500 mg/L), indicating the electro-enhanced system has been slightly effective on the bioaccumulation of lead (II) in the root part.

### Table 1. Accumulative biomass and water content (DW/FW) in the tissue of plant collected from each remediation method.

| Test no. | Experiment | Biomass (g dry wt.) | Ratio | DW/FWtot (3) | DW/FWshoot (4) | DW/FWroot (5) |
|----------|------------|---------------------|-------|--------------|----------------|---------------|
|          |            | Shoot (1) Root (2)  |       |              |                |               |
| 1        | EAPR 300   | 0.60                | 22.3  | 0.24         | 0.23           | 0.24          |
| 2        | EAPR 500   | 0.60                | 36.1  | 0.19         | 0.09           | 0.19          |
| 3        | Phyto 300  | 0.30                | 26.7  | 0.24         | 0.43           | 0.24          |
| 4        | Phyto 500  | 1.37                | 39.4  | 0.22         | 0.14           | 0.22          |

The following equations are to calculate the dry weight-fresh weight (DW/FW) ratio in total, shoot and root.

1. \[ (DW/FW)_{\text{total}} = \frac{1}{(1 + (2)/\text{total fresh weight (FW) of plant tissues (g wet wt.)}).} \]
2. \[ (DW/FW)_{\text{shoot}} = \frac{1}{\text{fresh weight of shoot (g wet wt.)}.} \]
3. \[ (DW/FW)_{\text{root}} = \frac{2}{\text{fresh weight of root (g wet wt.)}.} \]

Control plant was defined here as the plant grown in lead(II) concentration without urea.

The content of total chlorophyll and chlorophyll a/b ratio of plant (n = 3) on the effect of initial lead (II) concentration (Figure 6a, i.e. 300 and 500 mg/L of lead (II) concentration, represent as EP 300 or 500 and Phyto 300 or 500) and two differences of urea concentration (Figure 6b, i.e. 0.1 and 0.01 % of urea concentration, represent as EP 0.1 or 0.01 % and Phyto 0.1 or 0.01 %), respectively. Chlorophyll for control plant was defined here as the plant grown in nutrient solution without lead (II) concentration.
lead (II) in the root part rather than the bioavailability of lead (II) concentration in the plant tissue. However, the up-lifted lead (II) ions from contaminated water upward the rhizosphere was faster than the mechanism of plant to adsorb the lead (II) ions under the EP system [18], revealing the translocation of lead (II) ions from root to shoot was lower in the phytoremediation system (i.e. low TF and high BC values) than that in the EP system. In this regard, the effectiveness of EP system was encouraged by the plant growth as well as the biomass production which affected on the removal of lead (II) from water [11].

Figure 4b shows the addition of urea significantly increased the adsorption capacity of lead (II) ions by plant as shown in high TF and BC values. For phytoremediation, in 0.01% urea treatment, the amount of lead (II) concentration was much adsorbed in the plant root compared with 0.1% urea treatment. However, there was significantly difference results in the EP system between treated and untreated with urea. Generally, lower urea concentration increased the bioaccumulation of lead (II) concentration in the plant. For example, high BC value showed in the treated urea (i.e. BC = 5.43 for 0.1% and BC = 18.82 for 0.01%) than untreated urea (i.e. BC = 6.00 for EP 500), even though it was appeared in the same concentration of lead (II) (i.e. 500 mg/L). In this study, the addition of 0.1% urea increased the pH of solution up to mild alkaline, which a preferable condition on the adsorption of lead (II) ions by plant.

The EP system performed a high and rapid removal of the initial lead concentration from the contaminated water within 9d of testing period as shown in Figure 5. In the end of experiment, around 98% of lead (II) concentration from treated sample (i.e. 300 and 500 mg/L) has been successfully removed from contaminated water. While in the phytoremediation, the initial lead concentration was not significantly removed over the time as shown in Figure 5a, indicating the gradual saturation of adsorption capacity in the plant. Figure 5b shows high and rapid removal of 500 mg/L lead (II) concentration under the treated urea from contaminated water, revealing the chaotropic properties of urea has play an important role on the enhancement uptake by plant.

3.4. Lead-tolerance evaluation

Various factors such as chlorophyll content, biomass productivity, and water content were monitored during the EP system as well as the phytoremediation process to evaluate the lead-plant tolerance. Table 1 shows the biomass productivity (i.e. dry matter) and water content (i.e. DW/DF ratio) in the plant tissue. Under the stress conditions, dry to fresh plant weight (DW/FW) ratio showed as an indicator of water content in the tissue [40].

DW/FW ratio either in plant shoot or root (cols. 4 and 5) decreased by the increase of lead (II) concentration in the treated plant. Thus, high lead (II) concentration affects the decrease of water content in the treated plant. However, the addition of urea concentration (i.e. 0.1 % and 0.01 %) affected the DW/FW ratio of shoot and root (cols. 4 and 5) which showed a high and remain with the same level of control plant, except for test no. 5 where the ratio was in moderate level. Therefore, the additional of urea in the treated plant increased the water content in the plant tissues, suggesting the plant can grow well in contaminated water. Similar conclusion was also described in the application of EP system on the contaminated soil [15].

Biomass productivity of treated plant in the higher lead (II) concentration (i.e. 500 mg/L) has the same result with the plant grew in the lower lead (II) concentration (i.e. 300 mg/L) as well as the similar productivity with the level of control plants (col. 1). A substantial increase of biomass productivity was noted in addition of urea for each test, especially at low urea concentration (i.e. 0.01 % wt./v). These results suggested that the presence of urea in the EP system could sustain the healthy growth of treated plant in the contaminated water.

Chlorophyll content (i.e. total chlorophyll, and the Chl a/b ratio) had been determined as a parameter for photosynthetic activity as well as plant stress indicator. These parameters were assessed to evaluate the effect of contaminants exposure in the environment [40, 41, 42]. Figure 6 shows significant effect of lead (II) concentration on the content of total chlorophyll and chlorophyll a/b ratio.

Total chlorophyll content significantly decreased by EP system in high lead (II) concentration (i.e. EP 500), meanwhile content of total chlorophyll in phytoremediation had the same level as well as the control plant. High chlorophyll a/b ratio in all tests indicated that the plants were being exposed under chemical toxic showed less stress off [42]. In this regard the treated plant with low of lead (II) concentration (i.e. EP 300) was the most tolerant of chemical stressing as shown in Figure 6a. Under high concentration of urea (i.e. EP 0.1%), the plant could grow well in the EP system which showed high total chlorophyll content in the plant as shown in Figure 6b. Direct observation of phytomorphology changed on treated plant revealed that the plant did not show a significantly phytotoxic symptoms after 9d period of experiment (e.g., withering, yellowing, pigmentation, and discoloration), which indicated the plant had high tolerance of lead (II) concentration. Thus, the plant even though had under stress, but still able to maintain the relatively of normal photosynthetic pigments.

4. Conclusions

In conclusion, our study encourages a step ahead towards achieving the development an environmentally friendly of electro-enhanced phytoremediation (EP) technique on the removal of lead (II) from contaminated water. Several points from this study were highlighted as an option for metal contaminated water treatment in the future application.

1. The 2D electrode configuration had been applied in the EP system, the anodes were installed vertically from the cathode that placed in the center of tray allowing the current across the lead contaminated agar on the perimeter to maximize the acidic condition in the anode, while to minimize the alkaline condition in the cathode.
2. The lead (II) ions were migrated from anode to cathode, marked changes of lead concentration from the bottom toward the upper level of tray in the middle part of agar media which was showed by the V-shape. Therefore, the electrode configuration in this study can be applied effectively to encourage in the EP system on the rapid removal of lead (II) from the contaminated water.
3. Rapid removal of lead (II) concentration from contaminated water had been demonstrated by the EP system with Kentucky bluegrass (Poa pratensis L.) respected to all studied parameters. Bio-accumulation of lead (II) from contaminated water in the root part of hydroponic phytoremediation was higher than the EP system. However, the application of electro-assisted and addition of urea concentration (i.e. 0.1% and 0.01%) in the hydroponic plant increased the accumulation of lead (II) concentration in root and shoot part showed by high BC and TF value. Moreover, the presence of urea in the contaminated water increased the plant tolerance on the toxicity of lead (II) concentration.

Declarations

Author contribution statement

Rudy Syah Putra: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Shunitz Tanaka: Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data; Wrote the paper.
Wiyojo Priyo Wicaksono: Analyzed and interpreted the data; Wrote the paper.
Is Fatimah: Contributed reagents, materials, analysis tools or data.

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Declaration of interest's statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

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