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Assessing the Efficacy of Moringa, Neem, and Tulsi in Remediation of Sewage Water: A Comparative Study.

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ABSTRACT

The increase in a population's production and consumption habits causes an exponential rise in household waste, resulting in a lack of clean drinking water which leads to the main cause of water pollution. A cost-effective method is to use herbs as they are abundant in secondary metabolites. The purpose of this study was to understand how indigenous herbs can be utilized for treating sewage water, that can be used by citizens to get access to clean drinking water. A low-cost method was created to investigate the efficacy of herbs such as *Moringa oleifera* (Moringa), *Azadirachta indica* (Neem), and *Ocimum sanctum* (Tulsi), in the treatment of sewage water. Four combinations of herbs were selected, and the results were compared with municipal-treated water from a wastewater treatment facility. The samples were tested for parameters such as Estimation of Copper, pH, Chemical Oxygen Demand (COD), and Most Probable Number (Coliform). Coliform levels and copper levels in the herb-treated water were almost one-fourth the level as compared to sewage water. In both parameters, statistical significance was obtained. Statistical significance was considered at $p < 0.034$. The herb-treated samples showed a reduction in the COD and an increase in pH towards neutrality, as compared to the sewage water. In all combinations tested, the herbs were successful in improving the quality of water when compared to the sewage water as well as the municipal treated water. Hence, it can be concluded that herbs are a good natural resource that can be used for the treatment of sewage water, as they are easily available, and the method is sustainable.

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INTRODUCTION

For human survival, water is a crucial natural resource (Ugwu et al., 2017) With the increase in population, industrialization, and economic growth freshwater consumption has increased and that has resulted in the mismanagement of natural resources. Rapid urbanization, increased farming activity, pesticide use, soil degradation, high population density, and improper waste management are only a few examples of the factors influencing freshwater sources' quality. Water scarcity stems from poor quality water – a UNESCO perspective (Villaseñor-Basulto et al., 2018). According to the Central Pollution Control Board (2020), and the Ministry of Home Affairs (2011), India produces 60 m^3/p/a of wastewater and about 63% of India's wastewater is not treated before it is mingled with freshwater resources respectively. Untreated water contains higher levels of carbon, nitrogen, and phosphorus, which contaminate water supplies by causing eutrophication and a decrease in dissolved oxygen. In addition to endangered aquatic species, this disturbs the ecosystem's balance. Recovering the nutrients from wastewater not only lowers emissions and protects aquatic life, but also strengthens the nation's economy and sense of independence (Gowd et al., 2023).

Wastewater treatment is the process of eliminating impurities from domestic and industrial sewage. It consists of impurities that are physical, chemical, and biological. Wastewater treatment's primary objectives are to reduce pollutants and provide a disposable effluent without harming the environment (DR et al., 2021). To overcome these issues, naturally available plant-based materials have been used to treat the sewage water. The process of coagulation is crucial to the treatment of wastewater. Its use includes cleaning turbidity out of wastewater. It's a long-standing tradition where tiny pieces of drumsticks or drumstick powder were added to polluted water, after which the dirt particles would settle, an easy method to clear turbidity. Francis Kweku Amagloh and Amos Benang conducted an experiment that demonstrated the bio-coagulant effect of *Moringa oleifera* seeds (Amagloh & Benang, 2009). Physical-chemical processes like coagulation and flocculation, for example, have been found to reduce pollution and produce clean water for reuse (Delelegn et al., 2018).

The bio-coagulant used in this study, *Moringa oleifera* seeds, is the most crucial component in water purification. They have been used to treat wastewater because it is safe for humans and has no apparent disadvantages (Mehdinejad & Bina, 2018) (Alo et al., 2012). Bio-coagulants have the advantages of being affordable, easy to use, eco-friendly, and environmentally sustainable for developing nations. Compared to chemical coagulants, in which, the alkalinity of wastewater can be greater (Desta & Bote, 2021).

Another natural herb that helps in purifying water is *Ocimum sanctum* (Tulsi). It is one of the holiest and most revered of the numerous medicinal and health-giving herbs, giving it the title of "The Queen of Herbs", "The Legendary", and the "Incomparable One" of India (Maharjan, 2019). The herbal plant *Ocimum sanctum* has antibacterial properties against a variety of microbes in addition to possessing anticancer, anti-diabetic, and anti-ulcer properties (Sivaraja Bannari et al., 2012). It has been demonstrated that the Tulsi plant can prevent the toxic effects of industrial pollutants like copper sulfate, and other heavy metals too (Rana et al., 2022).

Neem is an important low-cost adsorbent that can help in wastewater treatment to a great extent due to its exceptional properties. Heavy metal ion removal from wastewater using neem leaf powder has shown excellent effectiveness (Hassan et al., 2018). Neem is also used as a natural adsorbent in the removal of copper ions from an aqueous solution (Al Moharbi et al., 2020). Parts of the neem plant exhibit an antibacterial function by inhibiting microbial growth and/or the capacity for cell wall breakdown (Alzohairy, 2016). When wastewater is dumped into rivers, fields, and other water bodies without being treated or just partially treated, it poses a major threat to public health and hygiene (Vikas et al., 2014). These properties prove that Neem, along with a combination of other herbs, can be an important component in adapting a simple technique to help purify untreated water.

MATERIAL and METHODS

Sampling Method

Five hundred ml of municipal treated water sample was collected from the Ghatkopar Wastewater treatment facility, managed by the Municipal Corporation of Greater Mumbai (MCGM), Mumbai, India. One liter of sewage water was collected from the same plant from the pre-treated water collection. Both samples were collected in plastic bottles and were stored at 4°C to prevent decomposition and ageing if any.

For the treatment process, both the fresh and dried forms of moringa seeds were used. The fresh *Moringa oleifera* seeds were extracted from drumsticks bought from the local market, and the dried seeds were bought from an agriculture-horticulture-based establishment. Fresh leaves of both Neem and Tulsi were used from the local market and plants grown locally respectively. The rest of the lab equipment, including glassware and chemicals, was provided by the Department of Life Sciences, Jai Hind College, Mumbai. The four combinations of herbs used for the treatment process were:

a. Moringa + Tulsi
b. Moringa + Neem
c. Tulsi + Neem
d. Moringa + Tulsi + Neem

The sewage water, the municipal treated water, and the four herb treatment combinations were divided into sets to be tested for different parameters such as Estimation of Copper, pH, Chemical Oxygen Demand, and Most Probable Number (Coliform). The entire setup was repeated twice.

Laboratory analyzes

Extraction Process

Method 1

The experiment was performed by two different methods. In the first set, 4g each of fresh Moringa seeds, Neem, and Tulsi leaves were macerated and added to 20 ml of untreated sewage water samples. The samples were incubated at room temperature for 5 days. The conical flasks were stirred every day.

Method 2

In the second set, mature dried Moringa seeds were used. The outer hard coat of Moringa seeds was removed manually, and ground using an electric mixer. 4g each of Neem and Tulsi leaves were ground similarly. The herbs were added to 20ml of the untreated sewage water sample, the flasks were shaken vigorously for 5 minutes and were incubated at room temperature for 2 days.

Estimation of Copper

Copper is an essential inorganic element for living organisms since it activates certain enzymes concerned with the oxidation process, particularly in plants. Copper in excess is, however, harmful to aquatic life. Pollution is through excess of fungicide, insecticide, and discharge of wastes from metallurgical and ceramic industries. The reaction of sodium diethyl thiocarbamate reagent with copper gives copper salt of diethyl dithiocarbamate, which is golden brown. The formation of this compound is one of the most sensitive methods for the estimation of copper and is unaffected by pH between the range of 5.7 and 9.2. Citric acid acts as a scavenger and chelates any other metal ion present. Liquor ammonia helps in maintaining the pH levels. The copper ions in the solution form a straw-coloured complex with carbamate which is extracted in the organic phase using isoamyl acetate or chloroform. Following separation, the samples' optical densities were measured with a colourimeter. (Bureau of Indian Standards, 1974) The amount of copper present was then determined using the following formula:

$$\text{Amount of copper} = \frac{\text{O.D of Sample}}{\text{O.D of Sample}} * \frac{\text{Concentration of standart}}{\text{Volume of sample}} * 1000$$

pH testing

The samples' pH was measured using pH strips, and the result was reported according to the visible change in colour.

Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) is a measurement of the amount of organic matter in a water sample that is capable of being oxidized by a potent chemical oxidant. As a gauge of the organic and inorganic components present in water bodies as well as in municipal and industrial wastes' susceptibility to oxidation, COD is a frequently used method (Yao et al., 2014). The amounts of organic contaminants in wastewater are often estimated using the COD metric. Hence the parameter was used to estimate the organic contaminants present in the water sample treated.

The estimation of COD was done by titration, where the sample is checked for the amount of iodine liberated, by titrating it against sodium thiosulphate using starch as an indicator. The amount of Iodine liberated is the amount of Oxygen reduced by Potassium iodide (Aini & Juwitaningtyas, 2022). The COD levels were estimated using the following formula:

Blank(A) = Burette reading for blank

Flask (B) = Burette reading for sample water (treated and non-treated water sample) (A-B) = Amount of $KMnO_4$ required for oxidation

$$\text{COD} = \frac{(\text{A-B})\ \text{ml} * \text{N}/80 * 8 * 1000\ \text{ml}}{\text{Amount of sample water}}$$

Most Probable Number (Coliform)

Total Most Probable Number (coliform) testing is one of the simplest methods for determining whether a water source is contaminated with bacteria. Total coliform counts provide a broad indication of a water's sanitation condition. The test was performed to understand and study the number of coliforms present per 100 ml of sample. MacConkey's broth of double and single strength was used. After incubating for 24 hours the sample was then analyzed and the results were noted using MacConkey's index (Ukpong & Udechukwu, 2015).

Statistical Analysis

All statistical analysis was performed in Microsoft Office Excel 2019 (version 2403). Data was analyzed using One-way ANOVA for the Estimation of Copper and Most Probable Number (Coliform). Post-hoc tests (Tukey's HSD) were performed using Real Statistics Resource Pack (XRealStats). The level of significance was set at 0.034. Statistical significance was considered at $p < 0.034$.

RESULTS

Extraction Process:

Extraction obtained by first methodology

The outcomes of the 5-day incubation process for all of the water samples altered in colour, and fungal development was noticed, but no explicit changes in the water, like coagulation or a reduction in turbidity were seen. As a result, the water was discarded without additional testing or inspection.

Extraction obtained from the second methodology

The results obtained using the mature Moringa seeds and the ground leaves were significantly different from those of the first approach. The sample showed obvious flocculation and coagulation. Hence, the samples were used for additional analysis of the estimation of heavy metals like copper, pH, Most Probable Number (Coliform), and COD.

Estimation of Copper

Results of testing the water for heavy metals i.e. copper, found in the sewage water, municipal treated sewage water, and the different combinations of herb-treated sewage water have been plotted as a bar graph (Fig. 1), to help with better comprehension of the various treatments and how they differed from one another. Error Bars exhibiting the Standard deviation are displayed. According to the data, there is a statistical difference in the reduction in the cooper levels when we compare the sewage water to the municipal treated sewage water and the different herb combinations used to treat the water. Statistical differences were observed (denoted as *). No significant difference was seen among the other groups. Statistical significance was considered at $p < 0.034$.

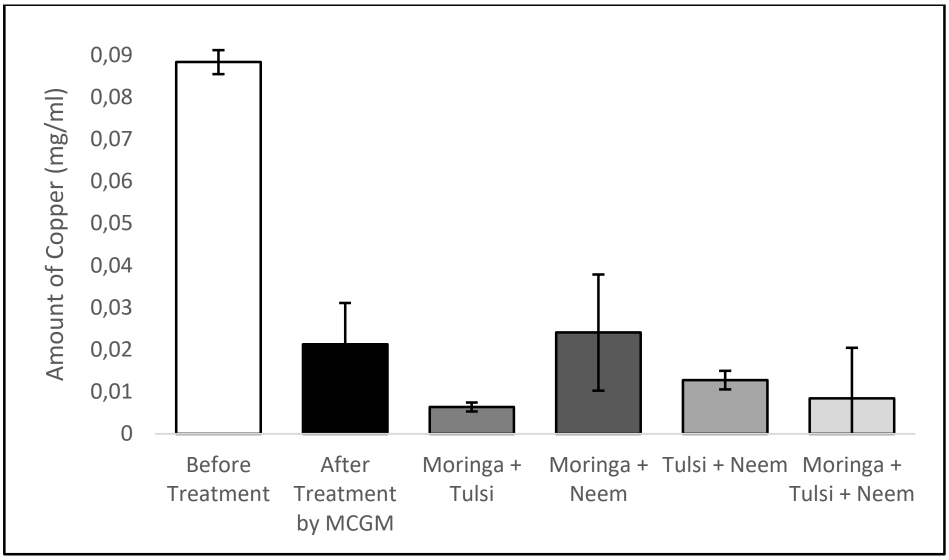

Figure 1. Bar graph displaying the amount of copper levels in sewage water, municipal treated water, and the combinations of herbs used to treat sewage water. The amount of copper in all combinations of herb-treated sewage water, and the municipal-treated sewage water showed a significant decrease when compared to the sewage water (denoted as □). No significant difference was seen among other groups when compared to each other.

pH Testing:

In many chemical, medicinal, and environmental monitoring activities, the determination of pH values is crucial (Kumar Dewangan et al., 2023). pH is a measurement of the proportion of free hydrogen and hydroxyl ions in water. Water with more free hydroxyl ions is basic, and water with more free hydrogen ions is acidic. Since chemicals in the water can modify pH, it is a crucial sign of a chemical change in the water. Hence testing of pH is necessary to understand the potential of Hydrogen ions in a sample.

The pH of the sample after the municipal treatment, and herb treatment was found to be approximately in the range of 6.0 to 8.0, indicating that it was neither too acidic nor too basic, and the results were plotted as a bar graph (Fig. 2). Additionally, when compared to the sewage water, the pH is one the lower side, indicating it is more acidic. Error Bars exhibiting the Standard deviation are displayed.

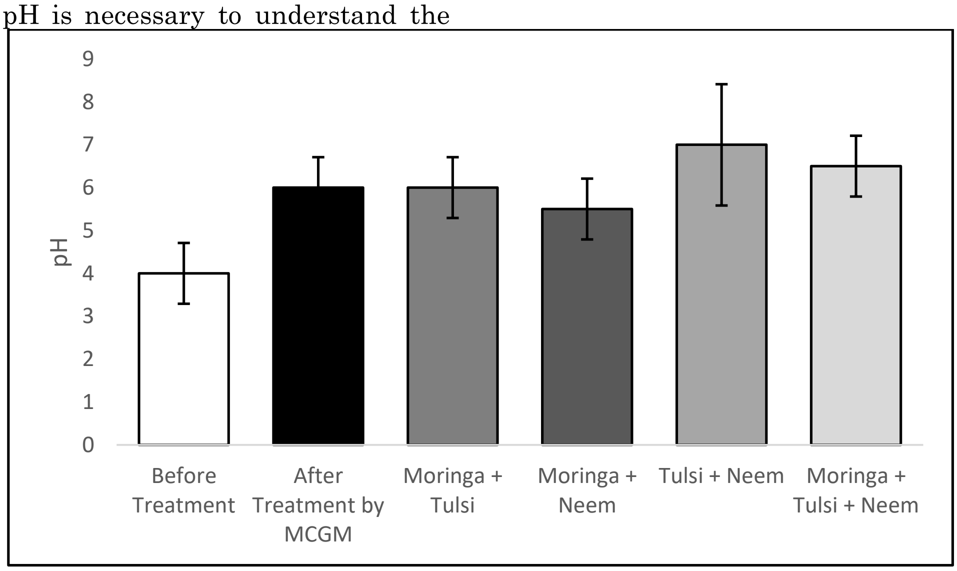

Figure 2. Bar graph displaying the pH range in sewage water, municipal treated water and the combinations of herbs used to treat sewage water. No significant difference was seen among other groups when compared to each other.

Most Probable Number (Coliform):

Compared to the sewage water, the Coliform levels in the municipal treated water decreased considerably. However, the sewage water treated with herbs had an even lower coliform level. The results when plotted in a bar graph (Fig. 3) give us a better understanding and comparison of the result. Error Bars exhibiting the Standard deviation are displayed. It shows that the herb-treated water is statistically significant when compared to coliform levels in the sewage water (denoted as □). No significant difference was seen among the other groups. Statistical significance was considered at $p < 0.034$.

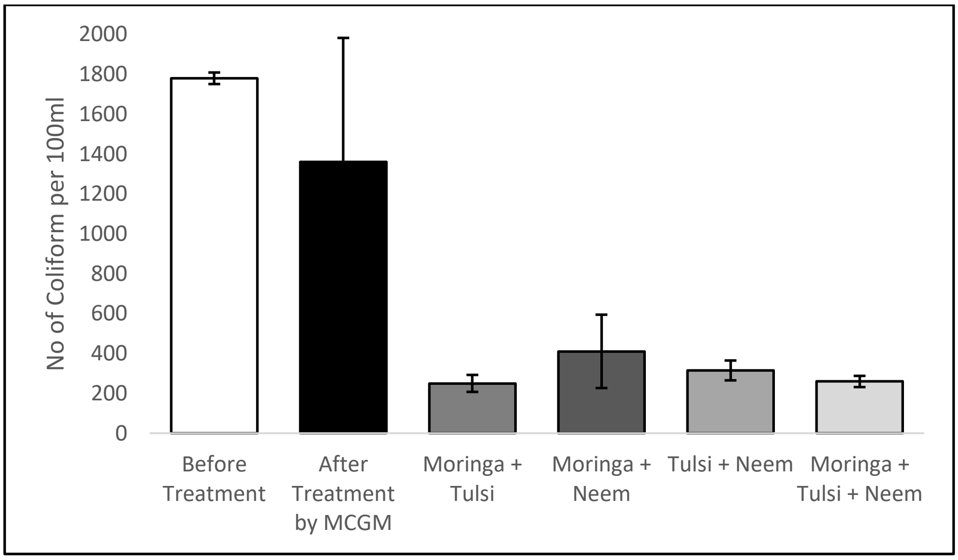

Figure 3. Bar graph displaying the MPN (Coliform) levels of sewage water, municipal treated water, and the combinations of herbs used to treat sewage water. The coliform levels in herb-treated sewage water showed a significant decrease when compared to the sewage water (denoted as □). No significant difference was seen among other groups when compared to each other.

Chemical Oxygen Demand:

The results obtained after titration represent the Chemical Oxygen Demand (COD) levels in mg/ml. The results obtained showed that the herb-treated water has considerably less COD compared to the municipal-treated water. The results have been represented in a bar graph (Fig. 4). Error Bars exhibiting the Standard error are displayed. The graph shows there is a comparative difference between the combinations of herb-treated water with municipal plant-treated water and sewage water.

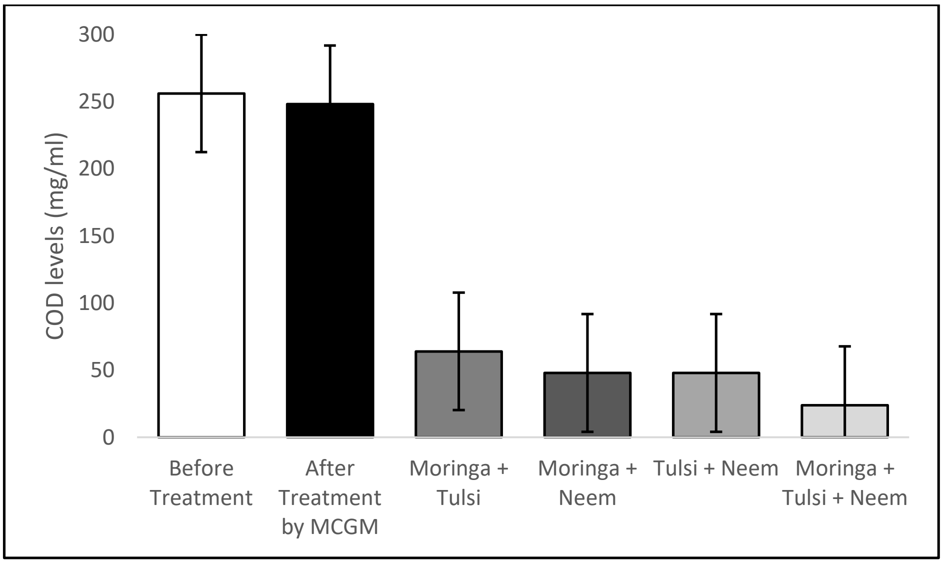

Figure 4. Bar graph displaying the COD levels difference in sewage water, municipal treated water, and the combinations of herbs used to treat sewage water. No significant difference was seen among other groups when compared to each other.

DISCUSSION

The aim of this study was to understand the effectiveness of natural herbs in treating sewage wastewater and compare the same with water treated at a municipal plant. This study found that *Moringa oleifera* seeds, a natural coagulant, and *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) are effective in purifying water. This was proved by examining the efficacy of these herbs on the Estimation of Copper, pH, Chemical Oxygen Demand levels, and Most Probable Number (Coliform) levels of the water, comparing them with water treated at a municipal treatment plant as well as the raw sewage water.

Copper is an essential inorganic element for living organisms, however, copper in excess is harmful to aquatic life as well as human life. Exposure to high levels of copper can lead to diarrhoea, hormonal imbalance, fatigue, nausea, and kidney problems. The Chemical Oxygen Demand measures the amount of oxygen required to oxidize organic and inorganic components in water. High levels of COD lead to reduced amounts of dissolved oxygen (required by aquatic life) which may lead to anaerobic conditions, causing the death of aquatic organisms. Coliform bacteria are commonly found in the digestive tract and are excreted in the faeces. They are often found in sewage and wastewater. A high coliform count in water indicates water contamination. The pH of water samples is an important regulatory test to confirm the portability of the water sample. Water with a pH of less than 6 is likely to be contaminated with pollutants making it unsafe to drink.

The results of this study showed that the herb-treated water had higher efficacy compared to municipal-treated water, with significantly decreased coliform and copper levels, reduced COD levels, and brought the pH levels close to neutrality. The study suggests that these natural herbs could be a cost-effective and efficient solution for treating sewage wastewater.

The costs of a treatment plant setup are high, and the amount of sewage generated in developing nations daily is also high. Herbs can be utilized to purify sewage water since they are more cost-effective and abundant in countries such as India, contributing to developing a cleaner environment. Although the experiment only utilized a small amount of water, the amount of wastewater that is disposed of every day is enormous, making it difficult to translate these results on an industrial scale. Hence, this method can be adopted for better efficiency when used in smaller units like groups of houses, colonies, municipal wards, etc. Further research needs to be done to find out whether this water can be recycled and used for agricultural and industrial purposes on a large scale.

CONCLUSION and RECOMMENDATIONS

With the increase in population, industrialization, and economic growth freshwater consumption has increased and that has resulted in mismanagement of the natural resources. According to the researchers, an exponential rise in household waste has been caused by improvements in the population's production and consumption habits. The main cause of water pollution is sewage, which is handled improperly and pollutes freshwater sources, specifically in metropolitan cities. Nearly 62% of wastewater in urban India remains untreated or partially treated, which further gets disposed of in natural water bodies. The usual treatment methods include the activated sludge process, oxidation ponds, aerated lagoons, and trickling filters. These methods need space, heavy equipment, and time, which drives up the cost of the treatment. The expense of the treatment process can be decreased by using natural coagulants. The indigenous medical system uses plants, which are abundant in secondary metabolites, to treat a variety of illnesses. This study investigated the properties of *Moringa oleifera*, *Ocimum sanctum* (Tulsi), and *Azadirachta indica* (Neem) in the treatment of wastewater. The results obtained from these herbs-treated water are compared with water treated at the treatment plants. It has been concluded that herbs are a good natural resource that can be used for the treatment of sewage water, which is cost-effective and easily available and a sustainable method of treating water.

This study conducted to test the effects of herbs on sewage water and municipal treated water shows promising results, however, it is a pilot study. To be introduced to the public on a larger scale in housing colonies and small-scale industries, different concentrations of the herb samples can be used to increase effectiveness. Replication of data is imperative and increasing the number of sets will help with advanced statistical analysis thereby increasing the validity of the positive results.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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