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Effect of *Strychnos potatorum* Linn. Seed Extracts on Water Samples from Different Sources and with Diverse Properties

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Abstract: In developing countries, ground water, which is contaminated with domestic and industrial waste, is commonly used for drinking. Colour, turbidity and microbial content affect quality of potable water. *Strychnos potatorum* (Linn.) seeds are known to function as coagulants and clarify water. Our study investigated the effect of the seed powder (10-40 mg L^{-1}) on water samples of varying colour, turbidity and microbial content. Samples studied were of river Yamuna, industrial effluent, a recreational pond, tap water and distilled water. pH was maintained within ±5 units while alkalinity was within one unit, showing that taste parameters were not affected. There was an 82-98% decrease in absorbance, denoting the seed's clarifying effect. The reduction in heterotrophic microbial load was 99% when 10 mg L^{-1} of seed powder was added. Ames test demonstrated non-toxicity of the seed at the concentrations tested. This simple method can help as an effective and affordable pre-treatment method and bring down the costs of secondary treatment.

Key words: *Strychnos potatorum*, coagulation, flocculation, polluted water, microbial count.

Introduction

In developing countries, ground water is preferred for drinking and other household purposes due to non-availability of suitable aquifers. Increasing urbanization and extension of the urban boundaries into the countryside coupled with industrial growth has led to increased discharge of pollutants via drains into rivers and lakes. In India, 90% of the household wastewater is discharged into water bodies without effective pretreatment, leading to levels of contamination beyond the acceptable limits. Rural population depends on this important source and it is imperative that it does not become a health hazard. Any reasonably priced water treatment method focussing on reduction in microbial load can help save costs towards protective maintenance.

The most common problem with quality standards of wastewaters is their colour and turbidity because of finely dispersed suspended and colloidal particles. Natural metallic ions, humus and peat materials, planktons etc. give colour to water while turbidity is caused by the presence of colloidal and suspended matter such as clay, silt, finely divided organic and inorganic matter, microorganisms etc. Water treatment methods involving coagulation and flocculation are effective in eliminating colloidal particles. Being cost effective and easy to apply, these are commonly used in household water treatment (http://www.who.int).

A coagulant neutralizes the electrostatic surface potential of the particles, which then stick together on

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rapid mixing, aggregate into large flocs and settle down. Subsequently they can be removed by filtration. Efforts have been made to identify natural products, which can be used as coagulants. Traditional knowledge has been tapped in this respect to bioprospect for suitable extracts from plants or other natural sources. They are economically well suited for rural areas, and easy to put into practice with no requirement of complex technical expertise. Ancient writings refer to the use of plant materials like the seeds of Nirmali tree, *Strychnos potatorum* Linn. as natural coagulant to clarify wastewater (Sen and Bulusu, 1962). The use of this seed for water treatment continues in villages of the states of Tamil Nadu and Maharashtra in India. Microbial reduction is one of the parameters for determining the success of water treatment techniques.

Coagulation-flocculation using lime is effective in reducing microbial load but only at pH 11, which can alter the water's taste, requiring further treatments. A 99% or greater reduction in plate count bacteria is considered effective, while most household methods achieve only levels of 90% or less. Laboratory and field studies of *S. potatorum* and *Moringa oleifera* have shown promising results for their use as coagulants (Dhekane et al., 1970; Folkard and Sutherland, 2002; Sutherland et al., 1990; Tripathi et al., 1976). Microbial reductions of about 50% and 95% have been reported for plate count bacteria and turbidity, respectively (Tripathi et al., 1976). The seeds contain a polysaccharide, a mixture of galactomannan and galactan in ratio of one to seven (Adinolfi et al., 1994) that can serve as coagulant in direct filtration of water, and effectively remove turbidity, bacteria and viruses from surface water (Babu and Chaudhuri, 2005). In addition, it is cost effective and easy to use and handle. However, there has been little effort to scientifically document their efficacy in reducing microbial count on water having different turbidities or pollution levels or on water obtained from different sources. Specific dosages, which can be used for purification of such water, have not been studied.

Our present work shows that water obtained from diverse sources and with dissimilar properties can be clarified effectively using this seed. The samples tested were water from river Yamuna, effluent water from an industrial estate and water from a pond maintained at a popular tourist spot. These were compared with samples of distilled and tap water. River Yamuna receives effluent water from industries and household wastewater, and in its course through Delhi, gets maximally polluted (50%). It has shown highest faecal coliform levels of all rivers in India with an MPN value ranging from 3.7×10^6 to 5.2×10^6 at different places in Delhi (http://www.cpcb.nic.in). Water samples from this river, from the recreational pond area and industrial water, were our primary targets to check for any encouraging and desirable change as any effective preliminary treatment of these water sources can serve to make them potable at overall lowered costs. The parameters measured were pH, colour, alkalinity and microbial load. Analysis of pH and alkalinity change can demonstrate if there is any severe modification to the property of the water in terms of taste. Colour was measured since any change adversely affects its desirability for drinking purposes. Microbial colony forming units (CFU) were assessed to check their percent reduction after addition of the seed. Ames test was carried out to assess the toxicity of the seed powder.

Materials and Methods

Water samples were collected in autoclaved bottles from five sources: Pond water (PW) from a pond maintained at a popular tourist spot, Yamuna water (YW) from river Yamuna flowing near New Delhi, water from industrial effluent (IW), household tap water (TW) and distilled water (DW) from our laboratory. After authentication, the seeds were ground into fine powder using a mixer-grinder and different dosages of seed powder were added to standard amounts of the samples as mentioned below for different experiments. Standard dosages ranged from 10-40 mg L^{-1}. Colour (at 585 nm), pH, alkalinity and microbial count determinations were carried out on ten replicates for each sample per experiment and the experiments were repeated thrice.

Preparation of Samples

S. potatorum seeds were crushed into fine powder using a household grinder and the powder was then passed through a sieve to obtain particles of uniform size. Different dosages of the seed (0, 10, 20, 30, 40 mg L^{-1}) were added to the respective water samples. The samples were vortexed, initially for two minutes and then subjected to low speed stirring for approximately 20 minutes. After one hour, the samples were carefully decanted into another clean conical flask. Further analysis was performed using these water samples.

Determination of Water Characteristics

The pH was measured using electronic pH meter (Cyberscan) after calibration. Colour of the samples was measured at 585 nm (Systronics UV-Visible Spectrophotometer). Colour tests are generally measured at 425 and 580 nm to account for brown colouration and then assessments made. However, in samples YW and IW, maximum absorbance was recorded at 585 nm. We wanted to test for any decrease in absorbance after addition of seed coagulant at the maximally absorbed wavelength.

Alkalinity was measured according to the standard method suggested by Environmental Pollution Authority of the United States (http://www.epa.gov). The values were expressed as alkalinity equivalent to mg L^{-1} calcium carbonate ($CaCO_3$).

For establishing microbial load levels, 100 µl of each water sample was spread on petri plates containing nutrient agar (in g L^{-1}: 5.0 peptone, 3.0 beef extract and 8.0 sodium chloride) solidified with 1.5% agar. The plates were incubated at 37°C overnight and the number of visible colonies was counted the next day.

Ames test was carried out using *Salmonella typhimurium* strain TA 102 (His^-) according to the standard protocol (Levin et al., 1982). Two concentrations (10 mg L^{-1} and 40 mg L^{-1}) of the seed were tested. Sodium azide (0.5 µM) served as the positive control. Presence of colonies in a medium lacking histidine denotes that the test substance is toxic and has induced mutation in the organism leading to His^+ revertants. Absence of colonies indicates that the test substance has not induced mutation and is non-toxic.

Statistical Analyses

The results were subjected to Analysis of Variance (ANOVA) using the statistical programme COSTAT and Student's T-test to test for statistically significant variations ($P \leq 0.05$ level) for the parameters measured. Correlation and regression analyses were performed to test for the pattern of effect of seed dosage on water samples. Scatter plots were analyzed to study the pattern of variation of data obtained.

Results and Discussion

pH Level

With varying concentrations of seed extract, the samples maintained their pH level within ±0.5 unit (Figure 1), showing that the extract does not contribute to drastic changes in pH. In IW also, pH levels were maintained at the acidic range of 1-2 only. The correlation coefficient (r) was not significantly different at $P \leq 0.05$. Scatterplot study showed an increase in pH for increasing concentrations of seed until 20 mg L^{-1} after which it decreased. The pH of all samples (except IW) was in the range specified by WHO and Indian Standards (http://hppcb.nic.in) for potable quality of water (6.5-8.5) and was in the range of 7±1 unit.

Absorbance

The change in absorbance values was statistically significant for PW, YW and IW (Figure 2). There was an exponential decrease in absorbance after addition of seed dosage of 10 mg L^{-1}. PW showed a 98% decrease, while YW and IW showed 82% decrease. The regression statistics were insignificant at $P \leq 0.05$, suggesting a non-linear pattern of change in absorbance. DW and TW did not show absorbance at 585 nm. Such relatively colourless water samples have their λ_{max} at other wavelengths. The intention being to study clarification

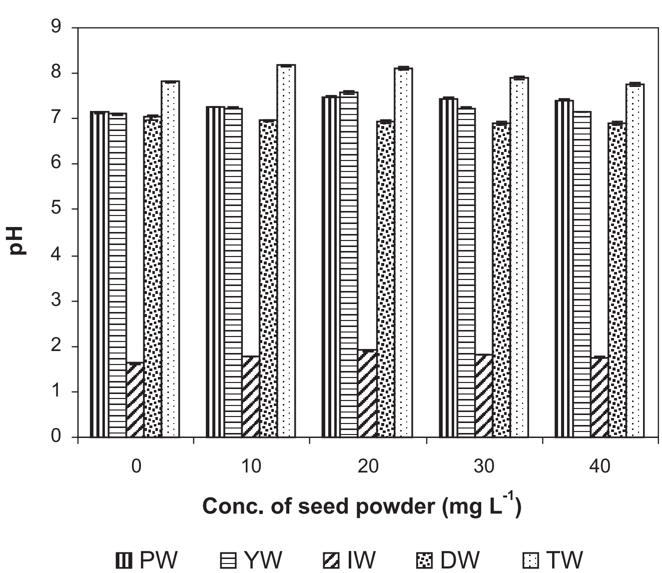

Figure 1: Effect of different dosages of *S. potatorum* seed powder on pH of water samples.

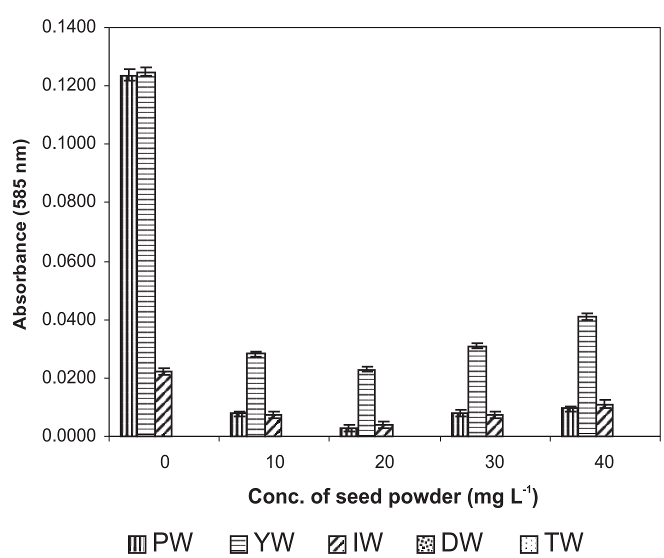

Figure 2: Effect of *S. potatorum* seed powder on absorbance of water samples at 585 nm.

of water samples, PW, YW and IW served the purpose of testing for this premise. It is notable that at seed concentrations greater than 20 mg L^{-1}, absorbance of YW increases, which could be due to the lingering presence of the seed particles, which did not flocculate effectively. Though IW maintained an acidic pH, the notable decrease in absorbance points to the efficient flocculating ability of this seed.

Alkalinity

The alkalinity increased until 20 mg L^{-1} (Table 1) and decreased at subsequent dosages. These were statistically significant (except PW). However, the distinct inference that emerges is that 20 mg L^{-1} is the noteworthy treatment level for all the samples, after which further increase of dosage does not change the alkalinity remarkably. Therefore, alkalinity, that can potentially alter the taste of water, does not change beyond one unit. The negative alkalinity values of IW indicate that the sample has an excess of strong acids, which further depress the pH (http://www.umass.edu).

Heterotrophic Microbial Count

The heterotrophic microbial count of all samples showed a significant decrease with the various treatment levels (Table 2). There was an exponential decline in the total count for all samples except IW, which did not show presence of microbes in control also. This could be due to its extremely acidic pH of 1, which did not support microbes. Probably usage of other media formulations or media at lower pH levels may have led to the detection of acidophiles. However, in order to maintain standard conditions for all treatment levels, these media were not tested and could be the subject of other experiments, since it has been shown that the seed itself does not drastically vary the pH. A ninety-nine percent reduction of CFUs was noted at the very first treatment level (10 mg L^{-1}), after which subsequent treatments brought them down to levels of 30-1100 CFU ml^{-1} for the various samples. An earlier report mentions the efficacy of treatment of surface water, with an initial CFU of 280-500 CFU ml^{-1}, decreasing to 5-20 CFU ml^{-1} (Babu and Chaudhuri, 2005). This alludes to a reduction level of 40% with a dosage of 1.5 mg L^{-1} of seed coagulant, which is within the potable water quality standards. We have used a higher dosage level, which were more effective. Higher dosages are necessary, as the initial CFU counts in our samples were much higher.

Toxicity

As *S. potatorum* has long been in use for household treatment purposes, it can be assumed that it is non-toxic since no adverse reports have been documented. Its components have been analyzed and shown to be

Table 1: Effect of *S. potatorum* seed powder on alkalinity of water samples

Concentration of seed powder (mg L^{-1})	*Alkalinity (mg L^{-1} $CaCO_3$)*				
	PW	*YW*	*IW*	*DW*	*TW*
0	0.0228	0.040	-0.448	0.002	0.055
10	0.0276	0.043	-0.412	0.003	0.058
20	0.030	0.048	-0.219	0.003	0.060
30	0.0258	0.046	-0.282	0.003	0.056
40	0.0259	0.044	-0.350	0.002	0.054

Data represent mean of 10 replicates for each sample with experiments repeated thrice. SD values were too small and hence not shown. Means within a column followed by the same letter are not statistically significant at $P \leq 0.05$ by One Way ANOVA.

Table 2: Effect of *S. potatorum* seed powder on heterotrophic microbial load (CFU ml^{-1}) of water samples

Concentration of seed powder (mg L^{-1})	*Microbial load (CFU ml^{-1})*				
	PW	*YW*	*IW*	*DW*	*TW*
0	499.4×10^2	507.2×10^2	0	152.43×10^2	312.54×10^2
10	9.99×10^2	11.57×10^2	0	7.55×10^2	9.46×10^2
20	0.56×10^2	0.89×10^2	0	0.489×10^2	0.736×10^2
30	0.54×10^2	0.80×10^2	0	0.349×10^2	0.653×10^2
40	0.51×10^2	0.74×10^2	0	0.307×10^2	0.596×10^2

Data represent mean of 10 replicates for each sample with experiments repeated thrice. SD values were too small and hence not shown. Means within a column followed by the same letter are not statistically significant at $P \leq 0.05$ by One Way ANOVA.

relatively harmless to the human system. In experimental animal models, aqueous extract and seed powder of *S. potatorum* were shown to be non-toxic upto a dosage level of 2000 mg kg^{-1} p.o. (Sanmugapriya and Venkataraman, 2006). However, to further establish toxicity aspects in the form in which the seed powder is administered generally, the Ames test was carried out. At the lowest and highest dosages used (10 and 40 mg L^{-1}), the coagulant did not trigger the formation of His^+ revertants of *S. typhimurium*, while revertant colonies were observed in the positive control sodium azide. Hence, the higher dosages tested can also be safely used.

Conclusions

Overall analysis of water quality parameters indicate that even samples showing heavy coloration and microbial load can be dosed with this seed for clarification purposes while maintaining the pH and alkalinity, thereby making the water potable. However, additional disinfection steps like boiling may be required for samples like PW, YW and IW to ensure optimal safety levels. The initial potentially detrimental features of these samples like colour and microbial load can be first ameliorated by use of the seed and subsequently treated with other methods. The advantage can be that less effort and resources would need to be put in after the initial clarification. It can lead to substantial savings in secondary treatment, leading to overall cost diminution. In a situation where water scarcity is a major problem, such household treatment methods offer hope for exploiting use of scarce water resources.

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