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Biosorption of Ni(II), Cu(II) and Pb(II) by *Punica geranatum* from Aqueous Solutions

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Abstract

In this paper removal of Ni(II), Cu(II) and Pb(II) ions from aqueous solutions by leaves of *Punica geranatum* were investigated. The biosorption was found to be pH dependent and the highest uptake of all the mentioned metal ions occurred at pH 4. Furthermore, the influence of other parameters such as initial metal ions concentration and contact time of biosorbent and sorbents were evaluated. Equilibrium data fitted very well to Langmuir model for all studied metals. It was also concluded that the Freundlich isotherm cannot be enough appropriate for the equilibrium data of all three metals. Biosorption of Ni(II), Cu(II) and Pb(II), reached equilibrium in 60, 60 and 30 min, respectively. Moreover, the adsorption rate of the metals can be best described by the second order model.

Keywords: Biosorption, *Punica geranatum*, Langmuir, Freundlich, Kinetic Models

1. Introduction

Development of industrialization and human activities culminated in the increased discharge of dreg and wastewater containing heavy metals into environment. The toxic effects of some metals, such as zinc, and the tendency of other metals, such as lead and cadmium, to accumulate within the food chain, make their presence in nature a severe threat to aquatic life [1].

The conventional techniques are commonly applied for the removal of heavy metals from wastewater including chemical (precipitation, neutralization, oxidation or reduction) or physical (ion exchange, membrane separation, reverse osmosis and adsorption processes) methods [2,3]. However, most of these methods are complicated and expensive. Furthermore, when these processes are applied to diluted metal wastes or lower concentrations of metal ions, they are ineffective [4,5].

Consequently, attempts have been made in order to find new straightforward and efficient techniques. Biosorption, the use of microorganisms (such as bacteria and fungi) and photosynthetic life (such as algae, aquatic and emergent plants), for the removal of metal ions, is one emerging technology that holds promise in this regard [6,7]. Considerable potential exists for these naturally existing, abundant and cheap sources of biomass [8]. The biosorption process is effective even if the concentration

is as low as 200 μg/ml [9].

There are two general mechanisms for the uptake of dissolved metal ions from water by biological biomass. The first one, metabolism-independent surface reactions, encompasses surface precipitation and surface complexation. This step is very rapid and occurs in a short time after the biomass comes into contact with the metals. The second one, however, is metabolism-dependent uptake step, in which the metal is transported in to the cells. This stage is slower than the first one [6,10].

Several mathematical models have been proposed in literature in order to describe the equilibrium isotherms of adsorption process. Langmuir and Freundlich isotherms, which are very well-known models in describing heavy metal biosorption, have been extensively in this work.

A general expression of the Langmuir model is [11,12]:

$$q_{eq} = \frac{q_{max}bC_{eq}}{1+bC_{eq}} \qquad (1)$$

where q_{eq} is the amount of metal adsorbed per gram of biomass weight at the equilibrium state, C_{eq} is the residual metal concentration left in solution after binding, q_{max} is the maximum possible amount of metallic ion, adsorbed per unit of weight of adsorbent and b is a constant related to the affinity of the binding sites for the metals.

The Freundlich model is expressed as [13]:

$$q_{eq} = K_F C_{eq}^{1/n} \qquad (2)$$

where q_{eq} is the amount of solute per weight unit of dry sorbent, K_F and n are empirically determined constants and C_{eq} is the solute equilibrium concentration. The Freundlich equation is often linearized by logarithmic transfer of Equation (2).

$$\ln q_{eq} = \ln k_F + \frac{1}{n} \ln C_{eq} \qquad (3)$$

The amount of metal ions adsorbed per unit of empty sorbent was obtained by using the following equation:

$$q = \frac{(C_i - C_e)V}{M} \qquad (4)$$

where q is the amount of metal ions, adsorbed onto the mass unit of the adsorbent, C_i and C_e are the concentrations of the metal ions before and after biosorption, V is the volume of the aqueous phase, and M is the amount of the adsorbent.

The kinetics of heavy metal adsorption can be modeled by the Lagergren pseudo-first order and second order reaction rate equations.

Lagergren developed a model for adsorption kinetics based on the first order reaction [14]. The equation is expressed as follows:

$$\ln(q_{eq} - q_t) = \ln q_{eq} - k_1 t \qquad (5)$$

where k_1 is Lagergren rate constant for adsorption, q_{eq} is the amount of adsorbate, adsorbed at equilibrium state and q_t is the amount of adsorbate adsorbed at any given time t.

A second order, reaction rate equation was proposed by Ho and Mckay to study the kinetics of adsorption [15]. The equation is expressed as follows:

$$\frac{t}{q_t} = \frac{1}{K_2 q_{eq}^2} + \frac{t}{q_{eq}} \qquad (6)$$

where K_2 is the second order adsorption rate constant.

In the present study the potential of *Punica geranatum* leaves for removal of Ni(II), Cu(II) and Pb(II) ions from aqueous solutions, and the effect of various parameters such as contact time of biosorbent and sorbents, pH of metal solutions and initial metal ions concentration have been investigated. Equilibrium modeling of biosorption was carried out using Langmuir and Freundlich, adsorption models equations. In addition, the biosorption Lagergren pseudo-first order and second order models were used to elucidate the kinetic characteristics of this process.

2. Materials and Methods

2.1. Preparation of Biomass and Metal Solutions

The leaves of *Punica geranatum* were collected in May 2006 from Yazd, Iran. The leaves were extensively washed with deionized water and then dried at room temperature. Dried biomass was powdered in a laboratory blender and sorted by sieving technique using the standard test sieves. The batch of biomass with suitable particle size (35-60 mesh) was selected for further experiments. The stock solutions (1000 mg/L) of Ni(II), Cu(II) and Pb(II) were prepared from analytical grade nitrate salts (Merck). The working solutions were made by diluting the stock solutions to appropriate volumes. The pH of each solution was adjusted, with diluted or concentrated HCl and NaOH solutions before mixing of biomass.

2.2. Experimental Conditions

Batch biosorption studies were conducted in 100 ml flasks by transferring 50 ml of metal solutions with the appropriate pH and concentration, and 0.5 gr of the biomass. The flasks were agitated on a shaker at 100 rpm for a predetermined sorption time period. After the incubation time, solutions were filtered and the metal ions concentrations were measured by shimadzu, AA-6800 model flame atomic absorption spectrophotometer equipped with Hallow Cathode Lamp and air acetylene burner.

3. Results and Discussion

3.1. The Effect of pH on Biosorption

The solution pH is one of the most important parameters affecting the biosorption process [16]. The effect of pH on metal uptake is shown in **Figure 1**. It was observed that the biosorption of Ni(II), Cu(II) and Pb(II) on *P. granatum* leaves increased with pH up to 4.0. Solution pH affects both cell surface metal binding sites and metal chemistry in water [17]. At low pH values, cell wall ligands are totally involved in H^+ and H_3O^+ ions, so repulsive forces make binding sites inaccessible for the metal ions. With an increase in pH, the negative charge density on the biosorbent rises which results in attraction between these negative charges and the metal ions and therefore in increasing the biosorption [18,19].

It was revealed that biosorption was very nominal in pH 2 (12.6%, 11.7% and 21.4%, for Ni(II), Cu(II) and Pb(II), respectively). It was also found that maximum uptake percent of all metal ions was in pH 4. An insignificant decline in biosorption could be observed in pH 6 due to the decrease in the metal solubility and precipitation of metal hydroxides.

3.2. Effect of Initial Metal Concentrations

The metal uptake mechanism is remarkably dependent
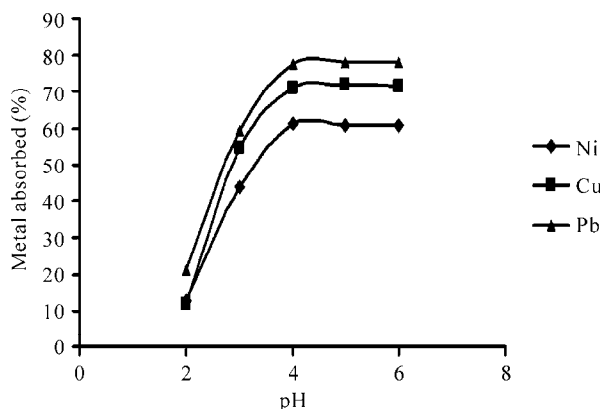
Figure 1. The effect of pH on the biosorption of Ni(II), Cu(II) and Pb(II) from 10 mg/L metal ion solution and 10 g/L *P. granatum* under equilibrium times and shake flask at 100 rpm at room temperature.

on the initial metal ions concentrations [20]. Increasing the initial metal ion concentration causes an increase in the biosorption capacity of the biosorbent which stems from the fact that the probability of collusion between metal ions and biosorbent increases in this condition which enhances the biosorption ability [18,21].

Sorption of heavy metals on *P. geranatum* biomass was carried out at diverse initial metal ion concentrations (5-1000 ppm) at pH 4. According to **Figure 2**, the slopes of the curves (q_{eq}) were enhanced in lower concentrations with increasing C_{eq} (equilibrium concentration) for all the examined metals and with further increment in concentration, q_{eq} values exhibited negligible variations due to the saturation of the binding sites.

3.3. Adsorption Isotherms

An adsorption isotherm is characterized by special constants which express the surface properties and the affinity of the sorbate to the sorbent [22]. Out of several isotherm equations, Langmuir and Freundlich models have been applied for this study, which were expressed previously in detail.

The linearized Langmuir and Freundlich adsorption isotherms of each metal ion are exhibited in **Figures 3** and **4**.

The Langmuir and Freundlich adsorption constants which were mentioned in Equations (1) and (2), together with the correlation coefficients (r^2) are listed in **Table 1**.

The r^2 values are respected as a measure of fitness of experimental data on the isotherm models [23], which for all the metals in the Langmuir model were very close to 1. The experimental maximum uptake values were 11.4, 15.5 and 18.4 for Ni(II) Cu(II), and Pb(II), respectively which showed acceptable accordance to theoretical values from the Langmuir equation. The q_{max} (maximum uptake) for Pb(II) was higher than other metals which
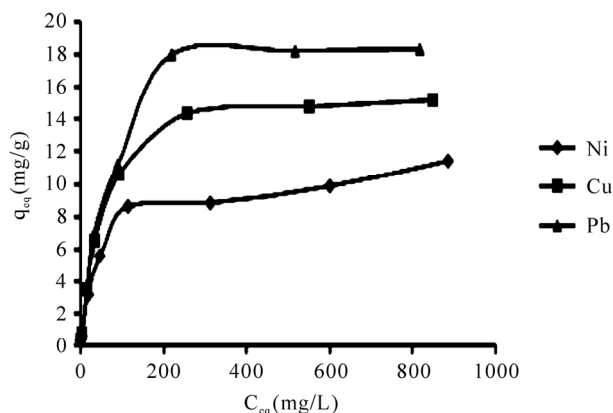
Figure 2. The effect of initial metal ion concentration (5-1000 mg/L), on the biosorption of Ni(II), Cu(II) and Pb(II), pH 4, 10 g/L *P. granatum* under equilibrium times in shake flask at 100 rpm at room temperature.
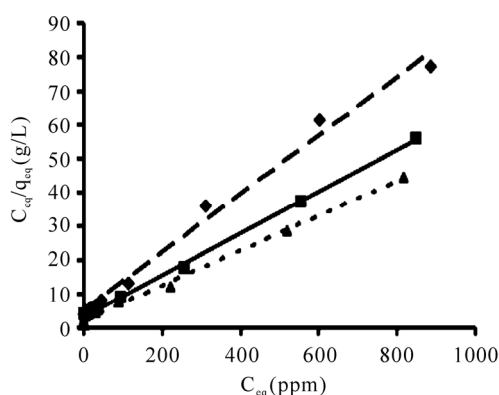
Figure 3. The Langmuir adsorption isotherms for Ni(II), Cu(II) and Pb(II) biosorption by *P. granatum* (10 g/L). Conditions: initial metal concentration of 5-1000 mg/L, pH 4, flask shaking at 100 rpm at room temperature under equilibrium time for each metal.
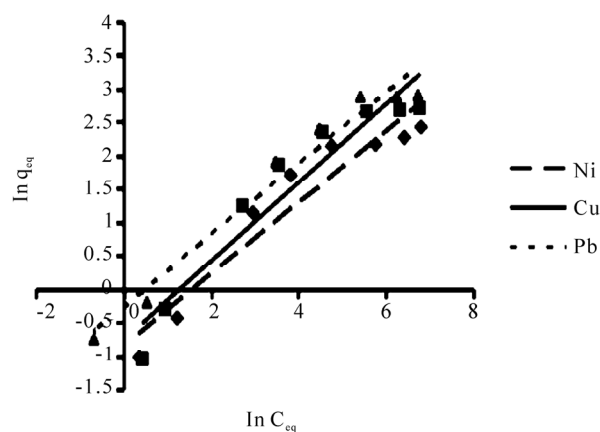
Figure 4. The Freundlich adsorption isotherm for Ni(II), Cu(II) and Pb(II) biosorption by *P. granatum* (10 g/L). Conditions: initial metal concentration of 5-1000 mg/L, pH 4, flask shaking at 100 rpm at room temperature under equilibrium time for each metal.

Table 1. Langmuir and Freundlich constants for the sorption of each metal with *P. granatum* biomass.

Metal ions	q_{max} (exp.)	Langmuir parameters			Freunlich parameters		
		q_{max} (theo.)	b	r^2	K_F	n	r^2
Ni	11.4	11.587	0.017	0.9908	0.437	1.873	0.915
Cu	15.5	16.207	0.018	0.9990	0.477	1.698	0.914
Pb	18.4	19.569	0.022	0.9958	0.779	1.875	0.964

implied the higher sorption capacity of *P. granatum* leaves for Pb(II). The relative order of metal uptake affinity of *P. granatum* leaves was Pb(II) > Cu(II) > Ni(II) based on *b* amounts.

The r^2 values for Freundlich isotherm were lower than Langmuir isotherm which expressed Ni(II), Cu(II) and Pb(II), ions adsorption are relatively conform to the Freundlich model, though not as perfect as to the Langmuir model.

3.4. Biosorption Kinetics

The change in the amount of adsorbed metal with time is shown in **Figure 5**. Biosorption of heavy metals demonestrated a fast rate during the initial 30 min due to huge amounts of unoccupied sites on the biosorbent. Biosorption of Ni(II), Cu(II) and Pb(II), reached equilibrium in 60, 60 and 30 min, respectively. Beyond these contact times there was no further increase in heavy metal removal because all the active sites had been occupied. The percent of heavy metal removal on the equilibrium times were 61.1%, 70.4% and 78.0% for Ni(II), Cu(II) and Pb(II), respectively.

According to the Equations (5) and (6) which were implied previously, kinetic data could not be described by the pseudo-first order model. Low correlation coefficients (< 0.68) were attained for this model in comparison with coefficients for second order model which were above 0.99 (**Figure 6**) for all the metals and acceptable fitness of experimental data in this model endorsed this results. It was proved from **Table 2** that the theoretical q_{eq} values calculated from Lagergren first order kinetic model for biosorption of Ni(II), Cu(II) and Pb(II), had huge variations with the experimental q_{eq} values, though the experimental and theoretical q_{eq} values for second order kinetic model can interpret more precise description of adsorption kinetic [13].

4. Conclusions

Punica granatum leaves was shown to be significantly effective in uptake of Pb(II) from aqueous solutions
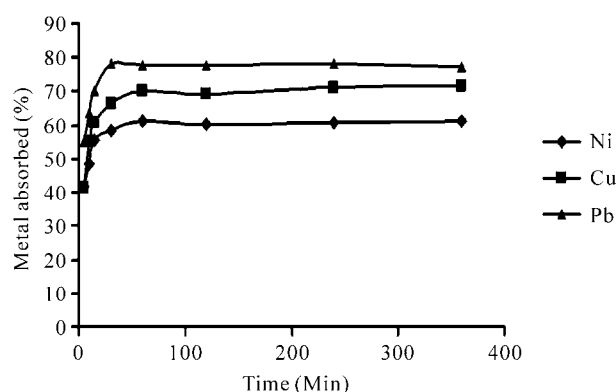
Figure 5. The time-course relationship of the biosorption of Ni(II), Cu(II) and Pb(II) from 10 mg/L metal ion solution, pH 4, 10 g/L *P. granatum* in shake flask at 100 rpm at room temperature.
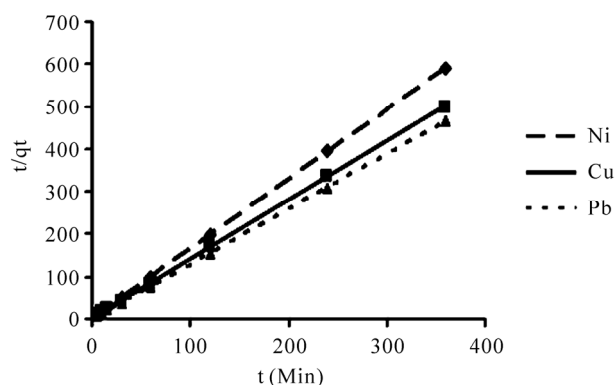
Figure 6. Biosorption of Ni(II), Cu(II) and Pb(II), by *P. granatum* on the second-order reaction kinetics model, as related to time (*t*) and the quantity of metal adsorbed at *t* (q_t).

Table 2. Pseudo-first-order and second-order kinetic constants for the sorption of each metal with *P. granatum* biomass.

Metal	q_{eq} (exp.) (mg/g)	Pseudo-first-order Kinetic constants			Second-order kinetic constasts		
		q_{eq} (theo.) (mg/g)	K_1 (1/min)	r^2	q_{eq} (theo.) (mg/g)	K_2 (1/min)	r^2
Ni	0.66	0.116	0.0031	0.4218	0.620	0.962	0.9998
Cu	0.74	0.135	0.0061	0.6739	0.724	0.414	0.9999
Pb	0.83	0.116	0.0030	0.3165	0.779	1.296	0.9999

among the tested metals which proves its notable application for remediation of environment from lead which is a threatening danger for environment and people's health. The study of metal's biosorption by *P. granatum* leaves indicated that the process conform Langmuir isotherm model, reasonably. The overall adsorption rate of the Ni(II), Cu(II) and Pb(II) can be best described by the

second order model which the results of experiments completely confirm this claim.

5. Acknowledgements

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Experimental Investigations and Theoretical Modeling Aspects in Column Studies for Removal of Cr(VI) from Aqueous Solutions Using Activated Tamarind Seeds

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Abstract

Continuous adsorption experiments are conducted using fixed-bed adsorption column to evaluate the performance of the adsorbent developed (from activated tamarind seeds) for the removal of Cr(VI) from aqueous solutions and the results obtained are validated with a model developed in this study. The effects of significant parameters such as flow rate, mass of adsorbent, and initial Cr(VI) concentration are studied and breakthrough curves are obtained. As the flow rate increases from 10 to 20 mL min^{-1}, the breakthrough time decreases from 210 to 80 min. As the mass of adsorbent increases, breakthrough time gets delayed. The breakthrough times are obtained as 110, 115 and 210 min for 15, 20 and 25 g of activated tamarind seeds. As the initial Cr(VI) concentration increases from 100 to 200 mgL^{-1}, the break point time decreases from 210 to 45 min. The process parameters for fixed-bed adsorption such as breakthrough time, total percentage removal of Cr(VI), adsorption exhaustion rate and fraction of unused bed length are calculated and the performance of fixed-bed adsorption column is analyzed. The mechanism for Cr(VI) adsorption on activated tamarind seeds is proposed. At low value of solution pH (= 1), the increase in Cr(VI) adsorption is due to the electrostatic attraction between positively charged groups of activated tamarind seeds and the HCrO^{4-}. A mathematical model for fixed-bed adsorption column is proposed by incorporating the effect of velocity variation along the bed length in the existing model. Pore and surface diffusion models are used to describe the intra-particle mechanism for Cr(VI) adsorption. The breakthrough curve obtained theoretically from pore diffusion model and surface diffusion model are compared with experimental results for different operating conditions. The standard deviation values obtained for pore diffusion model and solid diffusion model are 0.111 and 0.214 respectively.

1. Introduction

In all over the world, hexavalent chromium [Cr(VI)] has a dominant presence in most of the effluent streams as compared to other heavy metal ions. Cr(VI) compounds are used in various industries such as glass, ceramics, fungicides, rubber, fertilizers, tanning, mining, metallurgical etc [1-3]. Cr(VI) is produced as an effluent from the above mentioned industries which causes severe environmental and public health problems. Cr(VI) is highly mobile and is considered acutely toxic, carcinogenic and mutagenic to living organisms, and hence more hazardous than other heavy metals [4-7].

One useful solution for the elimination of Cr(VI) from the environment is the treatment of industrial effluent streams (wastewater) using an efficient method, adsorption, before being discharged into the aquatic systems [8,9]. Adsorption is an efficient and effective method for the removal of Cr(VI) when a suitable low cost adsorbent is identified.

Fixed bed adsorber is the most efficient arrangement for conducting adsorption process for industrial applications in wastewater treatment [10]. The design of an adsorption column depends on various important parameters such as flow rate, initial Cr(VI) concentration and bed height (mass of adsorbent). Understanding of ad-

sorption characteristics, determination of break point time for adsorption operation and effective utilization of the column is possible by carrying out the mathematical modeling of fixed-bed adsorption column.

Very few researchers have worked in the area of continuous adsorption studies for the Cr(VI) removal. Continuous adsorption studies are required to collect the experimental data for the design of adsorption column and for subsequent scale-up from pilot plant to industrial scale operation. Past studies mainly focused on analytical approach of solving the dynamics of fixed-bed adsorption column. The models, which are available, exclude some of the important physical aspects such as axial dispersion, intraparticle resistances and velocity variation along the bed length.

In the present study, activated tamarind seeds are used as a low-cost adsorbent for the removal of Cr(VI) from aqueous solutions. It is one of the biological waste products which is widely available in all over the world and can be easily cultivated in variety of soils. Continuous adsorption experiments are performed to see the effect of influencing parameters such as flow rate, bed height (mass of adsorbent), and initial Cr(VI) concentration on Cr(VI) removal. Mathematical model for fixed-bed adsorption column is proposed which takes account of both external and internal mass-transfer resistances as well as of non-ideal plug flow along the column. Mathematical model proposed is validated with the experimental results obtained in the present study and the results reported in the literature.

2. Materials and Methods

2.1. Adsorbent Preparation

Tamarind seeds are collected from the wastage of Cafeteria and Guest House (BITS-Pilani) of the institute. The seeds are washed with distilled water and dried at 110℃ for 5 h. The dried seeds are crushed into small particles by using Jaw crusher. Crushed seeds are sieved by 10-12 mesh BSS screens. The particles, having an average size of 1.85 mm, are treated with concentrated sulphuric acid (98% w/w) in 1:1 weight ratio and kept in an oven maintained at a temperature range of 150℃ for 24 h. The carbonized material is washed with distilled water to remove the free acid. Then it is soaked in 1% sodium bicarbonate solution for two days. The material is then washed with distilled water and dried again at 100℃ for 5 h [7,11].

2.2. Experimental Studies

A stock solution of 1000 mgL^{-1} of Cr(VI) is prepared by dissolving 2.8287 g of 99.9% potassium dichromate

($K_2Cr_2O_7$) in distilled water and volume of the solution is made upto 1000 mL. This solution is diluted as required to obtain standard solutions containing 20-800 mgL^{-1} of Cr(VI).

Continuous fixed-bed experiments are carried out to remove Cr(VI) from aqueous solutions using activated tamarind seeds. The schematic diagram of the experimental set up is shown in **Figure 1**. A glass column of 1.5 cm internal diameter is used as a fixed-bed column. The adsorbent bed is packed with the activated tamarind seeds in a stepwise procedure. Initially, two grams of adsorbent is filled into the column and is shaken manually in order to have dense packing. This procedure is continued till the whole mass of adsorbent is filled into the column. After this, 500 mL of distilled water is passed through the packed bed to make a more compact packing. Stock solution of Cr(VI) is passed in down flow mode through the fixed-bed and flow is controlled by a valve. To maintain the constant flow rate, constant level of Cr(VI) solution in overhead tank is maintained constant by pumping the solution from the tank containing stock solution of Cr(VI) as shown in **Figure 1**. The parameters varied in the present study are flow rate, mass of adsorbent (bed-height), and inlet Cr(VI) concentration. The flow rate is maintained constant using a liquid rotameter (0-15 L min^{-1}). The fixed mass of the adsorbent and the stock solution of initial Cr(VI) concentration is used to maintain the higher correctness in column experiments. The experiments are carried out at an optimum pH value of 1 obtained by batch studies [7]. The choice of this low pH is also based on the real pH value

1	Stored Cr(VI) solution
2	Outlet collection tank
3	Pump
4	Fixed-bed column
5	Feed of Cr(VI) solution
6	Outlet control valve
7	Inlet control valve
8	Base
9	Liquid Rotameter
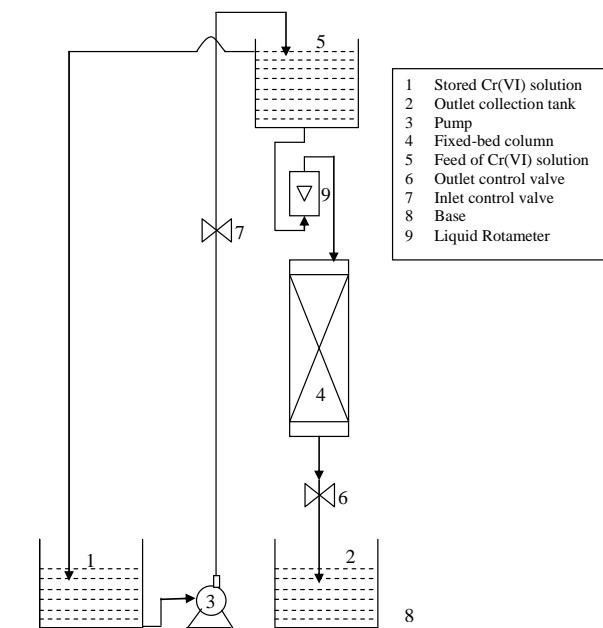
Figure 1. Fixed-bed continuous adsorption experimental setup for Cr(VI) removal from wastewater.

of industrial effluents such as chromium plating effluent (pH 1) [12], tannery effluent (pH 2) [13], and electroplating effluent (pH 2.2) [2], and the reported experimental studies in the literature [14,15].

In the present work, di-pheynl carbazide method is used to analyze the quantity of Cr(VI) ions in the aqueous solutions which only indicate the quantity of Cr(VI). This method has been reportedly used in a number of studies to analyze the Cr(VI) at low pH [3]. The concentration of Cr(VI) ions in the effluent is determined spectrophotometrically by developing a purple-violet color with 1, 5-diphenyl carbazide in acidic solution as a complexing agent [16]. The absorbance of the purple-violet colored solution is measured at a wavelength of 540 nm. The deviation of analytical method of Cr(VI) concentration is obtained by preparing the calibration curve. The standard deviation calculated for the calibration curve is 0.00453 which is an indicative of a good fit of the data and within the error limits of $\pm$ 1.64%. This ensured high confidence limits of the experimental analysis.

2.3. Mathematical Modeling and Simulation

In the present study, a mathematical model for the fixed-bed column is proposed by incorporation of an important parameter, the linear velocity variation along the bed, which affects significantly the design of an adsorption column [17]. The proposed model can be extensively used for understanding the dynamics of fixed-bed adsorption column for the adsorption of organic and inorganic (metal ions) compounds.

To formulate a generalized model for the fixed-bed adsorption column, following assumptions are made:

1) The adsorption column is operates under isothermal conditions.

2) The equilibrium of adsorption is described by Langmuir isotherm.

3) Mass transfer across the boundary layer surrounding the adsorbent particles is characterized by the external-film mass transfer coefficient, k_f.

4) The linear velocity of the bulk phase varies along the fixed-bed column.

5) The adsorbent particles are spherical and homogeneous in size and density.

Based on the above assumptions, the net rate of accumulation or depletion is given by Equation (1):

$$-D_L \frac{\partial^2 C_b}{\partial z} + V \frac{\partial C_b}{\partial z} + C_b \frac{\partial V}{\partial z} + \frac{\partial C_b}{\partial t} + \left(\frac{1-\varepsilon}{\varepsilon}\right)\rho_s \frac{\partial q_p}{\partial z} = 0 \tag{1}$$

The initial conditions are given by Equations (2) and (3):

$$C_b = C_{bo} \qquad z = 0, \ t = 0 \tag{2}$$

$$C_b = 0 \qquad 0 < z \leq L, \ t = 0 \tag{3}$$

The boundary conditions at both ends of the column are given by Equations (4) and (5):

$$D_L \frac{\partial C_b}{\partial z} = -V_0 \left(C_{bo} - C_b\right), \qquad z = 0, \ t > 0 \tag{4}$$

$$\frac{\partial C_b}{\partial z} = 0, \qquad z = L, \ t \geq 0 \tag{5}$$

The superficial velocity, *V*, in fixed-bed adsorption is not constant due to the adsorption of Cr(VI). For liquid phase adsorption, assuming the liquid density to be constant, the total mass balance is given by Equation (6) which is used to estimate the variation of velocity of bulk fluid along the bed height.

$$\rho_l \frac{\partial V}{\partial z} = -\left(1-\varepsilon\right)\rho_s \frac{\partial q_p}{\partial t} \tag{6}$$

Boundary conditions are given by Equations (7) and (8):

$$V = V_0, \qquad z = 0, \ t > 0 \tag{7}$$

$$\frac{\partial V}{\partial t} = 0, \qquad z = L, \ t > 0 \tag{8}$$

The transport of the solute species from the bulk of the fluid to the external surface of adsorbent particle constitutes an important step in the overall uptake process. For adsorption of solute in spherical pellets, the inter-phase mass transfer rate may be expressed by Equation (9):

$$\rho_s \frac{\partial q_p}{\partial t} = \frac{3k_f}{a_p}\left(C_b - C_e\right) \tag{9}$$

The intra-pellet solute transport mechanism is expressed by pore diffusion and solid diffusion and the associated adsorption isotherm for the system considered is Langmuir isotherm, which are discussed below.

2.3.1. Pore Diffusion

Intra-particle mass transport is characterized by the pore diffusion coefficient, D_P. The mass balance equation for the pore phase in a spherical particle can be written by Equation (10):

$$\varepsilon_p \frac{\partial c}{\partial t} + \left(1-\varepsilon_p\right)\rho_s \frac{\partial q_p}{\partial t} = D_p \left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r}\right) \tag{10}$$

The initial conditions considered are given by Equation (11):

$$c = 0, \quad q_p = 0, \quad 0 < r < a_p, \ t = 0 \tag{11}$$

The symmetry condition at the center of the adsorbent particle and continuity condition on the external surface of the particle are expressed by Equations (12) and (13), respectively:

$$\frac{\partial c}{\partial r} = 0, \qquad r = 0, \ t > 0 \tag{12}$$

$$k_{\mathrm{f}}\left(C_{\mathrm{b}}-C_{\mathrm{e}}\right)=D_{\mathrm{p}}\varepsilon_{\mathrm{P}}\frac{\partial c}{\partial r}, \quad r=a_{\mathrm{p}}, \ t>0 \tag{13}$$

2.3.2. Solid Diffusion

The mass balance equation for the intra-particle solute transport due to the solid diffusion phenomena is given by Equation (14):

$$\frac{\partial q_{\mathrm{p}}}{\partial t}=\frac{D_{\mathrm{s}}}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial q_{\mathrm{p}}}{\partial r}\right) \tag{14}$$

The symmetry condition at the center of the particles and continuity condition on the external surface of the adsorbent bed are expressed by Equations (15) and (16), respectivey:

$$\frac{\partial q_{\mathrm{p}}}{\partial r}=0, \quad r=0, \ t>0 \tag{15}$$

$$k_{\mathrm{f}}\left(C_{\mathrm{b}}-C_{\mathrm{e}}\right)=D_s\rho_s\frac{\partial q_{\mathrm{p}}}{\partial r}, \quad r=a_{\mathrm{p}}, \ t>0 \tag{16}$$

2.3.3. Adsorption Isotherm

The adsorption isotherm for the present system of Cr(VI) removal using activated tamarind seeds was found to be favorable and nonlinear, and is well described by Langmuir isotherm [7], and as given by Equation (17):

$$\theta=\frac{q_{\mathrm{e}}}{q_{\mathrm{m}}}=\frac{bC_{\mathrm{e}}}{1+bC_{\mathrm{e}}} \tag{17}$$

2.4. Simulation Algorithm

The preceding set of partial differential Equations (1) to (17) is solved numerically by reduction to set of non linear algebraic equations using the Explicit Finite Difference technique. Finite difference technique has been successfully applied to solve such type of partial differential equations in other studies by the authors group [17]. A mathematical algorithm to solve these coupled equations is developed and implemented into a computer program using MATLAB (v.6.1) software.

The standard deviation, s.d., was calculated using Equation (18) for comparing the model and experimental results and given as:

$$\mathrm{s.d.}=\sqrt{\sum\frac{\left(C_{\mathrm{exp}}-C_{\mathrm{model}}\right)^2}{N}} \tag{18}$$

3. Results and Discussion

The adsorption capacity of activated tamarind seeds as an adsorbent for the Cr(VI) removal was investigated in batch studies and reported in previous study [7,18]. The adsorbent capacity of activated tamarind seeds for Cr(VI) removal was also compared with other low cost adsorbent and found to be a potential alternative for removal of Cr(VI) from aqueous solutions. In the following sections, the effect of flow rate, mass of adsorbent and initial Cr(VI) concentration on the breakthrough curve for the removal of Cr(VI) by conducting the continuous column experiments is discussed. It also includes the validation of proposed mathematical model for fixed-bed adsorption column with the experimental results obtained in present study and the results reported in the literature.

3.1. Continuous Adsorption Experiments

Fixed bed column experiments are performed in order to generate the data for obtaining the breakthrough curves. The breakthrough time and the shape of the breakthrough curve are very important characteristics for the determination of dynamic response of the adsorption column. The breakpoint time is defined as the time of adsorption when the outlet concentration from the column was about 1-5% of the inlet concentration. The total stoichiometric quantity of adsorbed Cr(VI) (q_{t}, mg) in the column for a given inlet Cr(VI) concentration and flow rate is calculated from Equation (19):

$$q_{\mathrm{t}}=\frac{QA_{\mathrm{c}}}{1000}=\frac{Q}{1000}\int_{t=0}^{t=t_{\mathrm{total}}}C_{ad}\,dt \tag{19}$$

The area under the breakthrough curve (A_{c}) is obtained by plotting the adsorbed concentration (C_{ad}, mgL^{-1}) versus time (t, min). Total amount of Cr(VI) sent to column (m_{t}) was calculated from Equation (20):

$$m_{\mathrm{t}}=\frac{C_{\mathrm{bo}}Qt_{\mathrm{t}}}{1000} \tag{20}$$

where, t_{t} is the time equivalent to total stoichiometric capacity of the column which is defined by Equation (21):

$$t_{\mathrm{t}}=\frac{q_{\mathrm{t}}}{m_{\mathrm{t}}}t_{\mathrm{f}} \tag{21}$$

Total percentage removal of Cr(VI) is calculated from Equation (22):

$$\text{Total percentage removal of Cr (VI) } (S)=\frac{q_{\mathrm{t}}}{m_{\mathrm{t}}}\times100 \tag{22}$$

The empty bed residence time (*EBRT*) is the time required for the liquid to fill the empty column. The *EBRT* is given by Equation (23):

$$EBRT=\frac{\text{Bed volume}}{\text{Volumetric flow rate of the liquid}} \tag{23}$$

The adsorbent exhaustion rate (R_a) is the mass of adsorbent used (W) per volume of liquid treated at breakpoint time which is given by Equation (24):

$$\text{Adsorbent exhaustion rate } \left(R_a \right)$$
$$= \frac{\text{mass of adsorbent in column}}{\text{volume treated at breakthrough}} \quad (24)$$

The fraction of unused bed length is calculated from Equation (25):

$$y = 1 - \frac{t_b}{t_t} \quad (25)$$

Various parameters such as time equivalent to the total capacity of column (t_t), the total flow time (t_f), the breakthrough time (t_b), the total or stoichiometric amount of Cr (VI) adsorbed (q_t), the total amount of Cr(VI) sent to the column (m_t), the total percentage removal of Cr(VI) (S), the Empty Bed Resistance Time (EBRT), the adsorbent exhaustion rate (R_a) and the fraction of unused bed length (y) [19,20] are evaluated for Cr(VI) removal using activated tamarind seeds in fixed-bed adsorption column for different operating conditions (**Table 1**).

3.1.1. Effect of Flow Rate

The flow rate of wastewater stream flowing through the column plays a major role in designing an adsorption column. The breakthrough curves are obtained at different flow rates (10, 15 and 20 mL min⁻¹) by maintaining the constant adsorbent amount of 25 g and initial Cr(VI) concentration 100 mgL⁻¹ at a initial pH value 1 (**Figure 2**). As the flow rate increases, breakthrough time is obtained earlier. The breakthrough times obtained are 210, 115 and 80 min for flow rate values of 10, 15 and 20 mL min⁻¹, respectively. The total percentage removal of Cr(VI) in fixed-bed operations found to decrease from 50% to 45.1% with an increase in the flow rate from 10 to 20 mL min⁻¹ (**Table 1**). The total time, corresponding to

the stoichiometric capacity of the column, is found to be decreasing from 640 to 320 min with an increase in the flow rate from 10 to 20 mL min⁻¹ (**Table 1**). The fraction of unused bed length at breakthrough point increases from 0.67 to 0.75 with an increase in the flow rate from 10 to 20 mL min⁻¹. **Figure 2** shows that the steepness of breakthrough curve increases with increase in flow rate.

The decrease in breakthrough time with an increase in flow rate may be because of a fixed saturation capacity of the bed for the same concentration difference giving rise to a shorter time for saturation at higher flow rates. The increase in the steepness of breakthrough curve and the decrease in the removal efficiency (50% to 45.1%) with increase in the flow rate (10 to 20 mL min⁻¹) may be explained by the fact that when the residence time of Cr(VI) in the fixed-bed is not long enough for the adsorption equilibrium to be reached at that flow rate, the Cr(VI) solution leaves the fixed-bed before equilibrium occurs. Hence the contact time between Cr(VI) and activated tamarind seeds is very short at higher flow rates, results in a decrease in the removal efficiency (**Table 1**). Another probable reason for the faster saturation of the activated tamarind seeds bed at higher flow rates could be that with an increase in the flow rate, mixing increases and the thickness of the liquid film surrounding the activated tamarind seeds particle decreases, which reduces the film transfer resistance and hence an increase in the mass transfer rate.

3.1.2. Effect of Mass of Adsorbent

The shape of the breakthrough curve also depends on the mass of adsorbent (bed-length) used in the fixed-bed adsorption. The effect of mass of adsorbent is studied for 15, 20 and 25 g for activated tamarind seeds while maintaining the flow rate and the initial Cr(VI) concentration constant as 10 mL min⁻¹ and 100 mgL⁻¹, respectively at a pH value of 1. The breakthrough curves obtained for this study are shown in **Figure 3**. As the mass of adsorbent

Table 1. Different parameters for the Cr(VI) removal using activated tamarind seeds in a fixed-bed adsorption column for different operating conditions.

S No	C_{b0} (mgL⁻¹)	W (g)	Q (mL min⁻¹)	t_t (min)	t_f (min)	t_b (min)	q_t (mg)	m_t (mg)	S (%)	EBRT	R_a (g L⁻¹)	y
1	100	25	10	640	1280	210	640	1280	50.0	2.25	11.9	0.67
2	100	25	15	440	1000	115	690	1500	46.0	1.50	14.5	0.74
3	100	25	20	320	710	80	640	1420	45.1	1.12	15.6	0.75
4	100	20	10	430	930	155	430	930	46.2	1.80	12.9	0.64
5	100	15	10	310	780	110	310	780	39.7	0.76	13.6	0.65
6	150	25	15	300	920	100	675	2070	32.6	1.50	16.6	0.66
7	200	25	15	210	690	45	630	2070	30.4	1.50	37.0	0.78
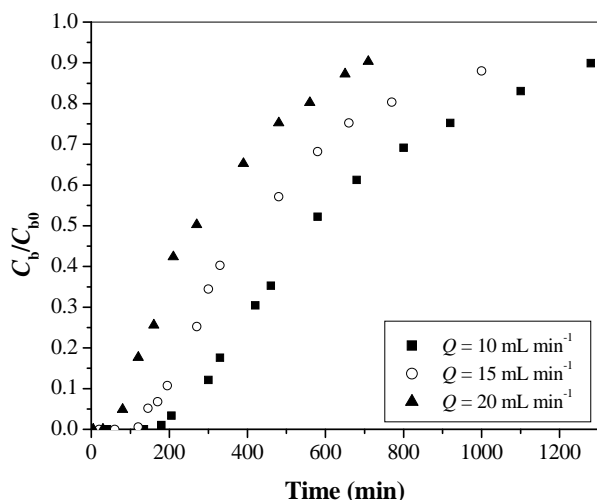
Figure 2. Effect of flow rate on breakthrough curve for Cr(VI) removal using activated tamarind seeds ($C_{b0} = 100$ mgL^{-1} and $W = 25$ g).
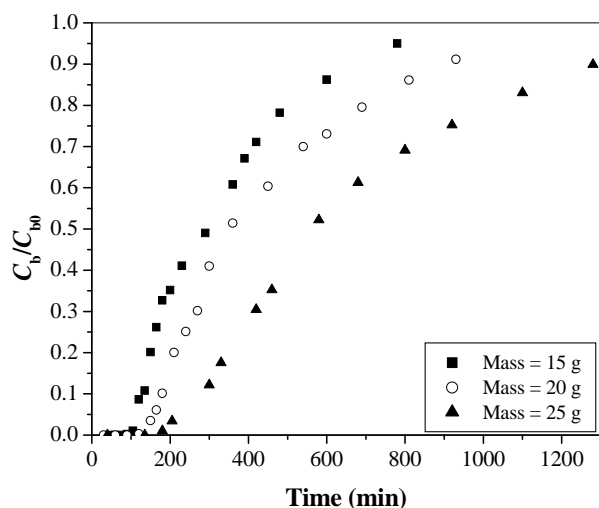
Figure 3. Effect of mass of adsorbent on breakthrough curve for Cr(VI) removal using activated tamarind seeds ($C_{b0} = 100$ mgL^{-1} and $Q = 10$ mL min^{-1}).

increases, breakthrough time gets delayed. The breakthrough times are obtained as 110, 115 and 210 min for 15, 20 and 25 g of activated tamarind seeds.

The total time, corresponding to the stoichiometric capacity of the column, is found to be increasing from 310 to 640 min with an increase in the mass of adsorbent from 15 to 25 g. (**Table 1**). The total percentage removal of Cr(VI) for the fixed-bed adsorption column increases from 39.7 to 50.0% with an increase in mass of adsorbent from 15 to 25 g (**Table 1**). The fraction of unused bed length at breakthrough point is found to be approximately same (within the range of 0.64-0.67) for 20 and 25 g of activated tamarind seeds. The rate of adsorbent exhaustion decreases from 13.6 to 11.9 g L^{-1} with an

increase in mass of activated tamarind seeds from 15 to 25 g.

Figure 3 shows that with an increase in the amount of activated tamarind seeds, the capacity of the fixed-bed adsorption column to adsorb Cr(VI) increases which results in a delay to obtain the breakpoint time. This may be due to the increase in the activated tamarind seeds surface area with increase in the amount of adsorbent which provides more binding sites for the adsorption of Cr(VI). For smaller bed-length, the rate of adsorbent exhaustion is higher which shows a faster exhaustion of the fixed-bed.

3.1.3. Effect of Initial Cr(VI) Concentration

In the adsorption of Cr(VI), a change in initial Cr(VI) concentration affects the operating characteristics of the fixed-bed adsorption column. The breakthrough curves obtained by varying initial Cr(VI) concentration from 100 to 200 mgL^{-1} for activated tamarind seeds are shown in **Figure 4**. As the initial Cr(VI) concentration increases from 100 to 200 mgL^{-1}, the break point time decreases from 210 to 45 min. The total time, corresponding to stoichiometric capacity of the column, is found to be decreasing from 640 to 210 min with an increase in the initial Cr(VI) concentration from 100 to 200 mgL^{-1} (**Table 1**). The total percentage removal of Cr(VI) for the fixed-bed adsorption column is decreasing from 50.0 to 30.4% with an increase in initial Cr(VI) concentration (**Table 1**). The fraction of unused bed length at breakthrough point is obtained in the range of 0.67 to 0.78 for 100 to 200 mgL^{-1} of initial Cr(VI) concentration (**Table 1**). The rate of adsorbent exhaustion increases in the range of 11.5 to 37 g L^{-1} with an increase in initial Cr(VI) concentration.
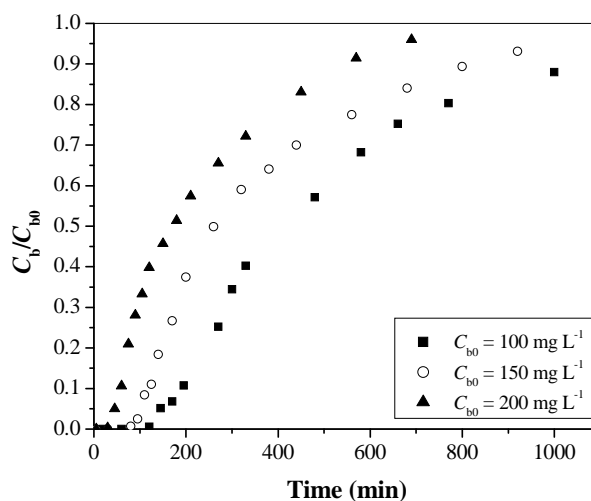
Figure 4. Effect of initial Cr(VI) concentration on breakthrough curve for Cr(VI) removal using activated tamarind seeds ($W = 25$ g and $Q = 15$ mL min^{-1}).

The increase in initial Cr(VI) concentration led to reach bed saturation earlier and obtaining breakthrough time quickly due to relatively slower transport due to a decrease in diffusion coefficient and decreased mass transfer coefficient at low Cr(VI) concentration [19]. Binding sites, quickly filled at higher initial concentration, result in a decrease in the breakthrough time. It is observed that the adsorbent get saturated faster at higher concentrations of adsorbate due to the higher rate of adsorbent exhaustion at higher Cr(VI) concentration. For a low initial Cr(VI) concentration, breakthrough occurs very late and surface of the adsorbents is saturated with Cr(VI) at a relatively longer time. This fact is probably associated with the availability of adsorption sites around or inside the adsorbent particles that are able to capture the Cr(VI) at lesser retention time.

3.1.4. Mechanism of Cr(VI) Adsorption

In adsorption, atoms, ions or molecules of a solute diffuse to the surface of adsorbent, where they either attach to the adsorbent surface due to the chemical bond or are physically held with weak intermolecular forces. The electrostatic charge and functional group interactions define the affinity of activated tamarind seeds for Cr(VI) adsorption. The Cr(VI) ions uptake on activated tamarind seeds mainly depends on (1) the Cr(VI) ions concentration, and (2) the adsorption and reduction phenomena that simultaneously take place on the activated tamarind seeds surface. The adsorption of Cr(VI) is more effective on acid-treated adsorbents [21]. In the present study, tamarind seeds are treated using concentrated H_2SO_4 at lower temperature (150℃). At this temperature, adsorbent surface generally develops acidic surface oxides and lower solution pH value. It is well documented in the literature that chemically activated naturally occurring adsorbent has a higher surface area and shows a larger capacity of Cr(VI) removal [6,21]. Cr(VI) ions adsorption on adsorbent surface is strongly depends to the solution pH. It can be related to the type and ionic state of the functional group present on the adsorbent surface [6,7,9].

The ionic state of the functional group on the activated tamarind seeds surface depends on the pH_{zpc} (zero point of charge) value of adsorbent. The value of pH_{zpc} of activated tamarind seeds is found as 5.4, and below this value of solution pH, the surface charge of the activated tamarind seeds is positive [22]. In the present study, the pH of the initial Cr(VI) solution is kept as 1 which is less than the value of pH_{zpc}. This indicates that the active sites of activated tamarind seeds are charged positively. For the solution pH range of 1.0 to 3.0, chromium ions can exist in the form of $HCrO_4^-$. At low value of solution pH 1, the increase in Cr(VI) adsorption is due to the electrostatic attraction between positively charged groups of activated tamarind seeds and the $HCrO_4^-$.

3.2. Mathematical Modeling and Simulation

In the present model, linear velocity variation along the bed is considered. The present mathematical model is simulated and validated using the parameter values reported by Bautista *et al.* [23] (**Table 2**). The present mathematical model is also compared with the experimental data of the same study with the model data obtained using present model and model proposed by Bautista *et al.* [23] as shown in **Figure 5**. The standard deviation (s.d.) is obtained as 0.113 for the results obtained with the present model and 0.139 for those obtained using Bautista *et al.* [23] model. The present model results show an improvement over the previous model with respect to better steepness of the breakthrough curve which is being validated by earlier studies [24,25]. This indicates that, consideration of effect of velocity variation along the bed length improve the existing model and is found to be better in explaining the trends obtained using experimental data. Based on the successful implementation of velocity variation in the model, and supporting evidence in the literature, simulations are carried out to validate the proposed model with the obtained experimental data for Cr(VI) removal using activated tamarind seeds in this study.

The comparison of experimental results and the model predictions (pore diffusion and solid diffusion models) for Cr(VI) adsorption using activated tamarind seeds is shown in **Figure 6**. Model parameter values for the simulation in the present study are listed in **Table 2**. The breakthrough curve obtained theoretically from pore diffusion model and surface diffusion model are compared with experimental results for different operating conditions and obtained standard deviation values are reported in **Table 3**. In the pore diffusion model a long tail is observed (**Figure 6**). But the solid diffusion model shows steeper breakthrough curve as compared to pore diffusion model. It may be due to the slow diffusion of Cr(VI) molecules into the center of adsorbent such as activated tamarind seeds [25]. The obtained experimental results are also found to be in agreement with the pore diffusion model (s.d. = 0.073 – 0.165) which confirms that the Cr(VI) adsorption on activated tamarind seeds can be described by pore diffusion mechanism (**Table 3**).

Figure 6 establish the fact that the experimental data for Cr(VI) adsorption using activated tamarind seeds fit well with the model initially but vary in the later half of the breakthrough curve. This may be due to the high fraction of unutilized bed length (0.64-0.78) which is responsible for more flattening of breakthrough curve at the latter stage of adsorption [25]. Langmuir isotherm model is found better than the Freundlich isotherm model to describe the equilibrium data for Cr(VI) adsorption on tamarind seeds. Langmuir isotherm is considered as favorable isotherm, whereas the Freundlich isotherm is

Table 2. Model parameters values used for simulation.

Parameter	Values (Bautista *et al.* [23])	Values (Present study)
ε, Bed porosity, (-)	0.58	0.3
ε_p, Particle porosity, (-)	0.53	0.2
ρ_p, Particle density, kg m^{-3}	1970	2000
ρ_b, Bed density$^\$$, kg m^{-3}	900	1110
ρ_l, Liquid density$^\$$, kg m^{-3}	1000	1000
D_L, Axial dispersion coefficient$^\$$, m^2 s^{-1}	5.9×10^{-10}	4.31×10^{-9}
q_m, Maximum adsorption capacity, mg g^{-1}	45.4	30.5
b, Langmuir isotherm constant, mL mg^{-1}	0.84	0.08
D_p, Pore diffusivity, m^2 s^{-1}	2.5×10^{-11}	8.62×10^{-9}
D_s, Surface diffusivity, m^2 s^{-1}	-	8.20×10^{-13}
k_f, External mass transfer coefficient, m s^{-1}	1.5×10^{-6}	3.5×10^{-5}
D, Bed diameter, m	0.016	0.015

($\$$) Assumed values based on the usual ranges reported in the literature [14]

Table 3. Standard deviation values for Cr(VI) adsorption on activated tamarind seeds by fixed-bed adsorption column on different operating conditions.

S No	C_0 (mgL^{-1})	W (g)	Q (mL min^{-1})	Standard deviation	
				Pore diffusion model	Solid diffusion model
1	100	25	10	0.107	0.331
2	100	25	15	0.114	0.155
3	100	25	20	0.102	0.198
4	100	20	10	0.073	0.136
5	150	25	15	0.106	0.192
6	200	25	15	0.165	0.277

accepted as a strongly favorable isotherm [25]. The unused bed length for favorable isotherm is higher than the strongly favorable isotherm [25]. Some of the model parameters (**Table 2**) are also assumed for the simulation of model in the present study. This may also one of the reasons for the obtained results (**Table 3**). Theoretically the steepness of breakthrough curve in solid diffusion model is more as compared to pore diffusion model in the latter half of adsorption [25]. Due to this reason, solid diffusion model is not found suitable for Cr(VI) removal using activated tamarind seeds.

4. Conclusions

A low cost adsorbent such as activated tamarind seeds is developed and found to be potential alternative for Cr(VI) removal from aqueous solutions. The breakthrough curves obtained by carrying out the fixed-bed columns experiments substantiates that the breakthrough curves
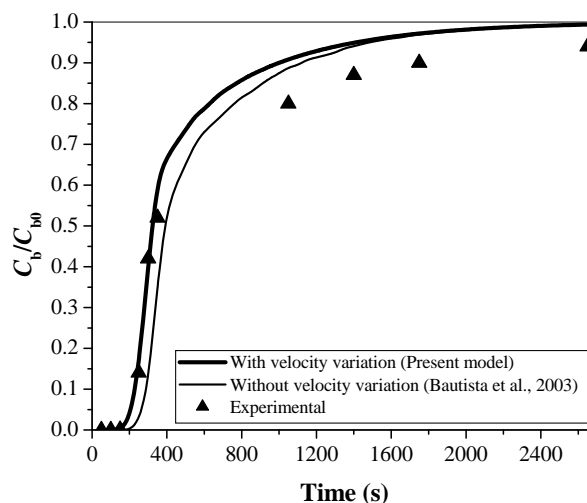
Figure 5. Comparison between theoretical and experimental results obtained using proposed mathematical model for the parameters given by Bautista *et al.* [23] (C_{b0} = 1000 mgL^{-1}, Q = 1 mL min^{-1} & W = 30 g).
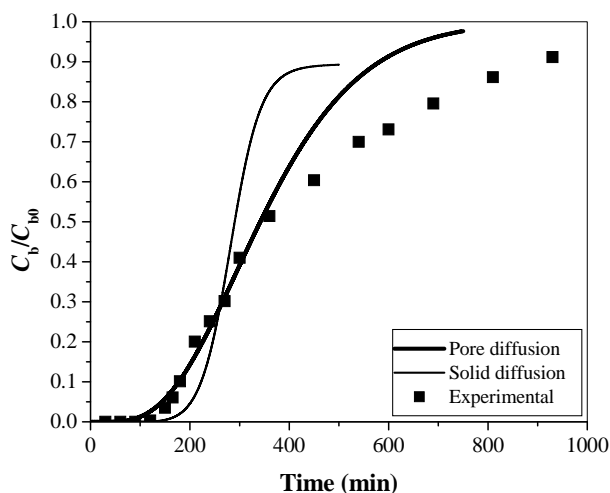
Figure 6. Comparison of experimental & theoretical break-through curve for Cr(VI) removal using activated tamarind seeds (Q = 10 mL min^{-1}, C_{b0} = 100 mgL^{-1} & W = 20 g).

are strong functions of adsorption exhaustion rate and fraction of unused bed length. It is observed that break-through point is obtained earlier by increasing the flow rate, decreasing the mass of adsorbent and increasing the initial Cr(VI) concentration. The proposed model is successfully validated with the literature data and obtained experimental data in the present study. Pore diffusion model is found suitable for explaining the breakthrough behavior attained for Cr(VI) removal using activated tamarind seeds. The results obtained from the proposed model show a good agreement with the experimental data for Cr(VI) adsorption during the initial time period of adsorption, but not so in the latter part of adsorption.

5. Acknowledgements

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Notation

a_P	Equivalent particle diameter, m
A_c	Area under the breakthrough curve, m^2
b	Langmuir isotherm constant, L g^{-1}
c	Adsorbate concentration inside the pellet, mgL^{-1}
C_{ad}	Concentration of Cr (VI) adsorbed, mgL^{-1}
C_b	Adsorbate concentration in mobile phase, mgL^{-1}
C_{bo}	Initial Cr (VI) concentration, mgL^{-1}
C_e	Concentration of Cr (VI) at equilibrium, mgL^{-1}
C_{exp}	Experimental Cr (VI) concentration in mobile phase, mgL^{-1}
C_{model}	Model values of Cr (VI) concentration in mobile phase, mgL^{-1}
D_L	Axial dispersion coefficient, m^2 s^{-1}
D_p	Pore diffusivity, m^2 s^{-1}
D_s	Solid diffusion coefficient, m^2 s^{-1}
$EBRT$	Empty bed residence time, s
k_f	External mass transfer coefficient, m s^{-1}
L	Bed height, m
m_t	Total amount of Cr(VI) sent to the column, mg
N	Number of data points, (-)
q_p	Average solid phase adsorbate concentration, mg g^{-1}
q_e	Equilibrium solid phase concentration, mg g^{-1}
q_t	Amount of adsorbate adsorbed at time, t, by the adsorbent, mg g^{-1}
q_m	Maximum monolayer adsorption capacity, mg g^{-1}
Q	Flow rate, mL min^{-1}
r	Radial distance, m
R_a	Adsorption exhaustion rate, g mL^{-1}
S	Percentage removal of Cr(VI) in fixed-bed adsorption column, (-)
$s.d.$	Standard deviation, (-)
t	Time, sec
t_b	Breakthrough time, sec
t_f	Final time of adsorption, sec
t_t	Time equivalent to stoichiometric capacity of the column, sec
V	Superficial velocity, m s^{-1}
V_o	Initial superficial velocity, m s^{-1}
W	Weight of adsorbent, g
y	Fraction of unused bed length
z	Axial coordinate, m

Greek Symbols

Θ	Fractional coverage, (-)
ε	Bed porosity, (-)
ε_p	Particle porosity, (-)
ρ_b	Bed density, kg m^{-3}
ρ_l	Liquid density, kg m^{-3}
ρ_s	Density of adsorbent, kg m^{-3}

◆◆ Scientific
◆◆ Research

Urban Wastewater Characteristic and its Management in Urban Areas—A Case Study of Mysore City, Karnataka, India

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Abstract

The rapid growth of the population, the technological and industrial boom has brought enormous problems and degradation of the environment. There is a gradual decline in availability of fresh water to be used for irrigation in India. As a consequence, the use of urban waste water (UWW) for irrigating agricultural lands is on the rise particularly in peri-urban areas of developing countries. Effective collection and treatment of urban wastewater is a critical problem in a developing country like India. A case study was undertaken to assess the characteristic of the urban waste water and its management in Mysore city and its long-term effect on irrigation. The untreated and treated urban waste water was collected during 2008 and analyzed in the laboratory. The suitability of the UWW for irrigation purposes is then evaluated according to the existing water quality standards and the results were compared with Food and Agriculture organization (FAO) irrigation water quality standards. It is evident from the results, that the current situation is not promising especially regarding the Electrical Conductivity, Total Dissolved Solids, Biochemical Oxygen Demand, Chemical Oxygen Demand, Suspended Solids and heavy metal concentrations and also pH of the treated UWW is exceeding the FAO standards. Among the heavy metals, the concentration of Iron and chromium are exceeding the FAO standards.

Keywords: Urban Waste Water, Water Quality Appraisal, Quality of Irrigation Water, Heavy Metal

1. Introduction

Expansion of urban populations and increased coverage of domestic water supply and sewerage give rise to greater quantities of urban waste water (UWW). On the contrary, providing safe and sufficient drinking water and proper sewerage system remains as the challenging tasks for many developing countries particularly so, in urban areas. With the increasing scarcity of freshwater resources that are available to agriculture, the use of urban wastewater in agriculture has increased, especially in arid and semi-arid countries. The major challenge is to optimize the benefits of wastewater as a resource of both the water and the nutrients it contains, and to minimize the negative impacts of its use on human health. About 80% of urban waste in India ends up in rivers where it destroys rivers ecosystems and it also makes bodies of water unfit for human use and if this trend continues the

rivers could soon become dead rivers.

UWW means domestic waste water, consisting of blackwater-excreta, urine and associated sludge and greywater-kitchen and bathroom wastewater or the mixture of domestic wastewater from commercial establishments and institutions including hospitals with industrial wastewater and run-off rain water [1] (**Figure 1**). Non point sources include silt from earth-moving activities; storm runoff from roads, home gardens, and industrial sites, infiltration from aquifers contaminated with domestic waste water or industrial chemicals; and automobile emissions. Domestic waste water created by residences, institutions, hospitals and commercial, industrial establishments, storm-water runoff from roads and other paved areas are frequently discharged into sewers. UWW is mainly comprised of water (99.9%) together with suspended and dissolved organic solids (carbohydrates, lignin, fats, soaps, synthetic detergents, synthetic organic
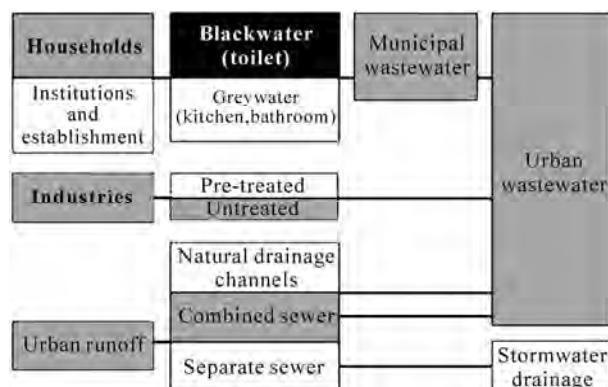
Figure 1. Urban waste water components.

chemicals from the process industries), inorganic solids including metals and also Pathogenic viruses and bacteria.

The fact is that, the UWW may contain toxic substances and discharge of untreated UWW results in contamination of water bodies which is harmful in many ways.

The sources of metal pollution in the UWW system can be classified into three main categories:

1) Domestic source.

2) Small scale industries (connected to the Waste Water Treatment System) and commercial.

3) Urban runoff.

The other principal sources of metals in UWW are body care products, pharmaceuticals, cleaning products, tanneries, metal plating units, jewellery industry and liquid wastes. Wastewater often contaminated with industrial effluent contains high levels of heavy metals. A large number of the cities/towns either do not have any sewerage system or system is overloaded or defunct. Even where sewers exist, they often leak or overflow, releasing their contents to storm water or other surface drains or percolate in to soil to reach ground water. Thus a bulk of pollution gets retained on land to percolate, leach or get washed-off to streams or groundwater.

All kinds of variations in wastewater use are possible and it is to be expected that different uses will have different impacts on agricultural productivity, the environment, and human health. Appropriate policy decisions and technical interventions are likely to depend on the nature and characteristics of the wastewater and the way in which it is being used. The following three types of wastewater use are the most relevant [1].

Direct use of untreated waste water is the application to land of wastewater directly from a sewerage system or other purpose-built wastewater conveyance system.

Direct use of treated wastewater is the use of treated wastewater where control exists over the conveyance of the wastewater from the point of discharge from a treatment works to a controlled area where it is used for irrigation.

Indirect use of wastewater is the planned application to land of wastewater from a receiving water body.

Irrigation with urban wastewater is a fact of life in and around urban areas in many low-income countries. Not only do the nutrients in the wastewater increase crop yields but also, there are adverse health implications, including bacterial contamination of vegetables and intensive application of pesticides to combat the insect pests that infest these crops [3]. Dietary intake of heavy metals has a substantial risk to the health, which is caused by consuming crops grown by using contaminated UWW [2].

Effective collection and treatment of UWW has become a very challenging task in most of the cities in India. There is a general practice of disposal of UWW into rivers, lakes or on land for irrigation with no treatment at all or with minimum treatment. The present study area Mysore city is having only primary treatment plant, which treats only a part of UWW generated from the city limits and large quantity of untreated UWW is discharged into low lying areas and water bodies located in and around Mysore City. Due to the scarcity of freshwater the untreated and treated UWW is used for irrigation. Hence it is necessary to determine the characteristics of the UWW in context to its utilization to irrigation and to evaluate the performance of the existing treatment plants. In the present study an attempt was made to know the nature and composition of the UWW in Mysore city and the changes in the composition after treatment with the special attention given to the concentration of heavy metal. The aim of the present study was to investigate the concentration of certain metals viz., cadmium, copper, lead, chromium, nickel, iron and zinc. The composition of urban waste water is influenced by the industries located within the area and urban runoff.

2. Materials and Methods

The study area Mysore is a unique city having around 12lakh population and was capital of former princely state of Karnataka. It lies between 12°9' and 11°6' latitude and 77°7' longitude and general elevation is little more than 1800feet above sea level. The climate of the city is moderated throughout the year with temperature during summer ranging from 30°C to 34°C. The rainy season is from May to October. The winter season is from November to February. The source of water for domestic and industrial purpose is mainly from the Cauvery River and ground water.

Mysore is one of the growing cities of Karnataka and it is so largely due to the presence of industrial resources and a well developed communication network. Mysore has a rich and vibrant history and heritage and hence attracts a huge number of tourists. Also, Mysore is now active center for production and industrialization. The

city has been growing as a counter magnet to Bangalore with large presence of software companies and the population is growing at a faster rate due to the influx of many industrial and commercial activities.

In recent years industrialization has become main cause of city's growth. There is diversity in industrial landscape of Mysore with haphazard distribution. The industrial areas are distributed all over the city and its surroundings with lack of order and regulation in industrial location. A large number of small medium and large scale industries exists in and around the Mysore city, including engineering, chemical, pharmaceutical, food, brewery, distillery, textile, steel and metal smelting.

Mysore has adequate water supply resources due to the proximity of Rivers Cauvery and Kapila. The city has four pumping stations namely Hongally Water Supply Scheme (I & II stage-8 mgd), Hongally Water Supply Scheme (III stage-12 mgd), Belagola Water Supply Scheme (11.5 mgd), Melapur Water Supply scheme (11 mgd quantity). The topography of the city is such that the entire UWW drains into three valleys viz., northern outfall into Kesare Valley, and other outfalls to the south one into Dalvai tank feeder valley and another to Malalavadi tank valley.

Mysore was one of the earliest cities in India to have underground drainage. In old parts of the city, underground drainage was completed in 1904. The quantity of domestic waste water generated has increased from 80 MLD during 2001 to 128 MLD during 2006-07. The area covered with sewer system is 100 Sq. kms and the total length of sewer lines is 740Kms. Only about 57% of the household are connected to under ground drainage system.

Based on the topography of the city, Mysore city comprises of five drainage districts, namely, A, B, C, D and E districts respectively, covering different areas (**Figure 2**). The city has been provided with three waste water treatment plants. For drainage districts A & D the waste water treatment plant of capacity 60.0 MLD is located at Rayankere, H.D.Kote Road, Mysore. The treatment plant for drainage district B is of capacity 67.65 MLD and it is located at sewage Farm, Vidyaranyapuram, Mysore. The treatment plant for drainage district C is of capacity 30.0 MLD and it is located at Kesare Village, Mysore. The waste water alongwith the waste water from point source and non point sources from different areas of the drainage districts are collected in wet wells and treated in the waste water treatment plants. All the treatment plants have facultative aerated lagoons and sedimentation basins. The process of primary treatment involves simple lagooning and subsequently natural oxidation where by the suspended colloidal particles of the UWW get partly or wholly coagulated and flocculated.

The fifth drainage district E in the city covers an area around 16 sq.kms. At present, this district does not have a sewerage system. There are several missing links at each district and 30 MLD is discharged as such on land, in low lying areas, which joins water bodies. The missing sewer lines identified in the above mentioned drainage districts are of varying diameters varying from 150 mm to 900 mm. The total length of missing sewer line identified as of 2002-03 is about 22135 meter. As of 2006-07, the quantity of sewage generated is 125 MLD and 95 MLD is treated. The untreated and treated UWW is largely used for irrigation.

Assessment of UWW characteristics was carried out during 2008, the untreated and treated UWW samples were collected in cleaned 2-L polythene bottles as per the standard methods from the following sampling locations;

Location No. 1: At Kesare Village, Mysore and waste water treatment plant No.1.

Location No. 2: At Sewage Farm, Vidyaranyapuram, Mysore and waste water treatment plant No. 2.

Location No. 3: At Rayankere, H.D.Kote Road, Mysore and waste water treatment treatment plant No. 3.

The samples were analysed to know the characteristics of physico-chemical parameters of the UWW as follows pH, Electrical Conductivity (EC), Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Total Dissolve Solids (TDS), Suspended Solids (SS), Sodium Absorption Ratio (SAR), Nitrate (NO_3^-N), Phosphate (PO_4^{3-}), Potassium(K^+).

3. Results and Discussion

The samples were analyzed for physic-chemical parameters by using standards methods [4]. The Presence of heavy metals in waste water is one of the main causes of water and soil pollution. The separate samples of untreated and treated UWW were collected for heavy metal analysis and preserved by adding 2 ml of concentrated nitric acid to prevent precipitation of metals and growth of algae. The samples were digested and concentrated 10 times from 100 ml to 10 ml on hot plate. Analysis of heavy metal content of the samples was performed using Inductively Coupled Plasma spectrometry (ICP). The results of physico-chemical parameters and heavy metal are presented in **Tables 1** and **2** respectively.

The pH range suitable for the existence of most biological life is quite narrow and critical, and is typically 6-9 [5]. High pH values above 8.5 are often caused by high bicarbonate and carbonate concentrations, known as alkalinity [6]. High carbonates cause calcium and magnesium ions to form insoluble minerals leaving sodium as the dominant ion in solution. Highly alkaline water can intensify sodic soil conditions, which will have implications for agriculture. The pH analysis of untreated UWW shows a maximum and minimum of 7.96 and 6.63 at location No. 2 during premonsoon and monsoon season

respectively. The pH of treated UWW treated UWW show a maximum of 9.11 at location No.1 and minimum of 7.48 at location No. 2 during post monsoon season. Slightly alkaline pH could be attributed to the detergents present in the UWW and in cases of acidity or alkalinity due to industrial effluent.

It is observed that the pH of untreated UWW is meeting the FAO irrigation water quality standards. The pH of treated UWW at treatment plants at location No. 1 & 2 during monsoon and post monsoon and at location no 3 during all seasons are exceeding the standards which may be attributed to the existing primary treatment process and treatment operational process. Higher pH over 8.5 may indicate other problems such as high sodium content. The pH of treated and untreated UWW is shown in **Figure 3**.
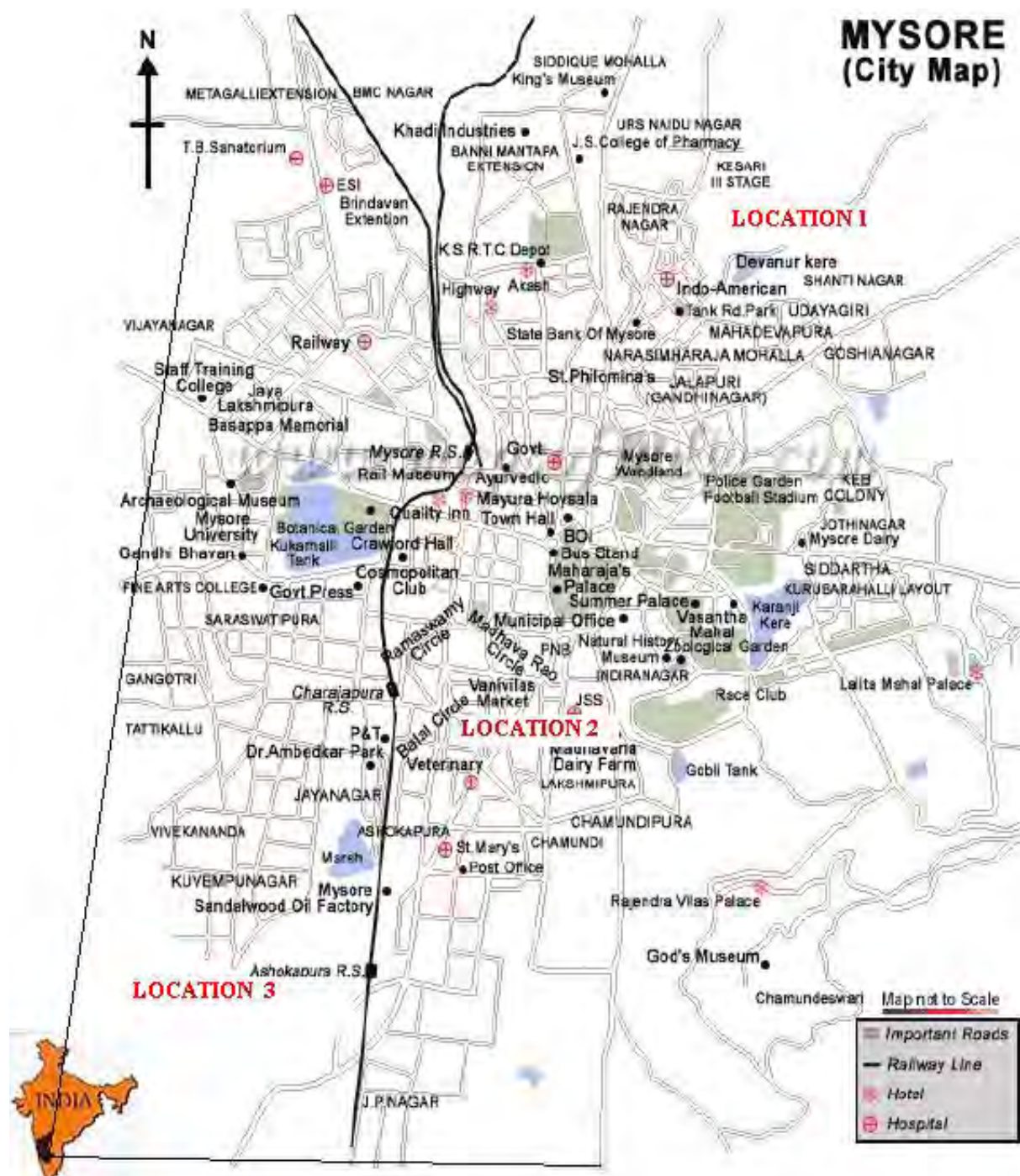
Figure 2. Drainage districts and wastewater treatment plants (Sample locations).

Table 1. Physico-chemical characteristics of UWW during 2008.

Sl.No	Parameters	Pre monsoon						Monsoon						Post monsoon					
		L1		L2		L3		L1		L2		L3		L1		L2		L3	
		Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated
1	pH	7.31	8.21	7.96	8.14	7.41	8.87	7.02	7.81	6.63	8.51	6.85	8.58	7.45	9.11	6.85	7.46	7.05	8.84
2	E.Cond (dS/m)	1.317	1.064	1.072	0.875	1.232	1.016	0.96	0.825	0.916	0.742	0.934	1.423	1.12	0.964	1.201	0.994	1.315	1.511
3	COD (mg/l)	235.0	163.0	295.0	52.0	139.0	36.0	159.0	105.0	175.0	89.0	140.0	132.0	445.0	75.0	488	83	494	106.0
4	BOD (mg/l)	125.0	45.0	141.0	15.0	15.0	9.0	12.0	7.0	17.0	6.0	10.0	5.0	300.0	18.0	370	45	260	10
5	TDS (mg/l)	780	600	600	520	680	580	600	530	580	500	540	860	720	620	780	680	800	980
6	SS (mg/l)	100	20	160	20	40	20	140	40	100	60	80	40	360	60	200	20	140	20
7	SAR	4.6	4.76	3.98	4.52	4.3	4.54	1.65	1.84	1.22	1.24	1.08	6.2	1.72	1.95	1.99	1.89	1.87	3.69
8	Nitrate-N (mg/l)	0.04	0.3	0.09	0.28	0.15	0.23	0.28	1.12	0.2	0.61	0.186	0.257	0.4	0.17	0.16	0.07	0.11	0.32
9	Phosphate (mg/l)	26.4	15.45	13.41	19.90	18.37	2.33	10.32	0.62	7.7	1.69	11.08	0.38	17.9	3.52	12.36	16.39	12.33	0.4
10	Potassium (mg/l)	46	42	44	40	40	41	13	18.6	7.1	8.3	5.1	3	31.8	16.8	16.8	17.6	18.8	102

Table 2. Heavy metal concentration of UWW in different seasons of 2008 in ppm.

Sl.No	Heavy-metal	Premonsoon						Monsoon						Post monsoon					
		L1		L2		L3		L1		L2		L3		L1		L2		L3	
		Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated
1	Cd	0.19	0.19	0.18	0.18	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.20	0.19	0.19	0.19
2	Cr	0.18	0.18	0.18	0.20	0.19	0.18	0.22	0.22	0.22	0.21	0.22	0.23	0.22	0.20	0.21	0.20	0.24	0.22
3	Cu	0.08	0.06	0.10	0.06	0.06	0.05	0.05	0.04	0.11	0.05	0.05	0.03	0.15	0.03	1.93	0.03	0.08	0.02
4	Fe	0.89	0.48	1.51	0.63	1.68	0.77	2.04	0.80	1.92	0.62	2.20	1.94	6.38	1.00	5.66	0.94	7.97	3.14
5	Ni	0.58	0.53	0.53	0.42	0.56	0.23	0.31	0.33	0.30	0.31	0.38	0.26	0.76	0.49	0.68	0.58	0.52	0.40
6	Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
7	Zn	0.17	0.14	0. 27	0.06	0.09	0.07	0.09	0.19	0.18	0.14	0.09	0.05	1.34	0.24	0.71	0.18	0.36	0.23

3.1. Biochemical Oxygen Demand (BOD)

BOD is the most widely used parameter to measure water quality and to design the waste water treatment plants. The determination of BOD involves the measurement of the dissolved oxygen (DO) used by microorganisms in the biochemical oxidation of organic matter, and is a measure of organic pollution.

BOD in the untreated UWW is high as of the organic content in the city wastewater is usually high. In the present study, BOD of untreated UWW ranged from 10 mg/l to 370 mg/l during dry season and the BOD of treated UWW show a range from 5.0 mg/l during monsoon to 45 mg/l during dry season. During dry seasons, the BOD of untreated UWW exceeds the CPCB Standards. After biological treatment in the Lagoons, BOD is reduced and is very much within the CPCB standards. Continuous irrigation and high organic matter contents may clog soil pores; however, this usually does not occur unless BOD levels exceed 500 mg/l [7]. The BOD of treated and untreated UWW is shown in **Figure 4**.

3.2. Chemical Oxygen Demand (COD)

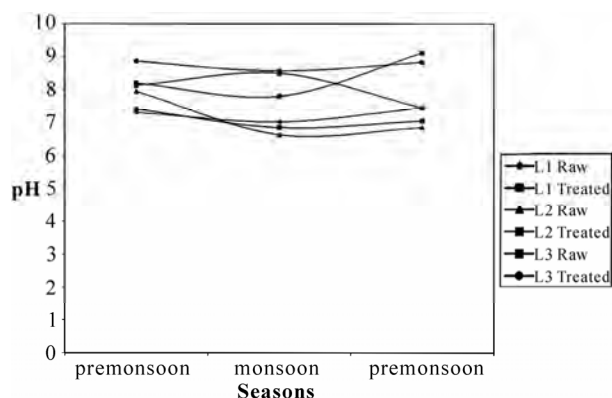The COD test is used to measure the oxygen equivalent of the organic material in wastewater that can be oxidized
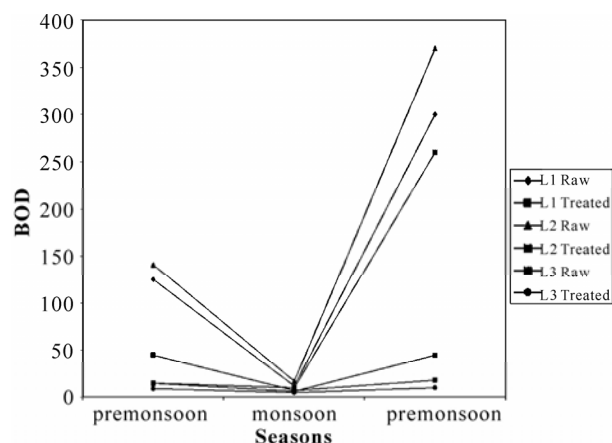
Figure 3. pH of treated and untreated UWW.

Figure 4. BOD of treated and untreated UWW.

chemically using dichromate in acid solution. COD in UWW is due to the presence of organic matter present in sewage mainly in the form of food waste.

In the present study COD of untreated UWW ranged from 139 mg/l during pre monsoon to 494 mg/l during post monsoon. Also treated UWW show a range from 36 mg/l to 163 mg/l during pre monsoon season. COD of untreated and treated UWW during monsoon is well within the CPCB inland surface water standards. However the COD of untreated UWW at location No. 2 during pre monsoon and at all the locations during post monsoon seasons are exceeding the standards. However after the biological treatment in the lagoons, the COD of the treated UWW at all treatment plants is very much reduced and well within the standards. The primary treatment process has reduced the BOD and COD concentrations. Chandrasekaran *et al.* [8] also reports the same findings. The COD of treated and untreated UWW is shown in **Figure 5**.

3.3. Electrical Conductivity (EC) and Total Dissolved Solids (TDS)

Electrical conductivity (EC) is a measure of the ions

present in water, the conductivity increases with the increase of ions. It is also effectively a surrogate for total dissolved solids (TDS) and is important for irrigation because it is a measure of the salinity of the water. The conductivity test does not identify the dissolved salts or the effects they may have on crops or soil, but it does indicate fairly reliably the degree with which a salinity problem is likely to occur. Salinity restricts the availability of water to plants by lowering the total water potential in the soil. Salinity also has an impact on crop physiology and yield with visible injury occurring at high salinity levels. Usually crop yield is independent of salt concentration when salinity is below some threshold level then yield gradually decreases to zero as the salt concentration increases to the level which cannot be tolerated by a given crop. Rice is a crop which is moderately sensitive to salt.

In the present study EC of untreated UWW show a range from 0.96 dS/m during monsoon to 1.31 dS/m during pre monsoon. EC of treated UWW showed a range from 0.74 dS/m during monsoon to 1.51 dS/m during post monsoon EC values of untreated and treated UWW at all the three locations lie within the slight to moderate range of the FAO irrigation water quality standards.

TDS of untreated UWW in the present study show a range of 540 mg/l during monsoon 800 mg/l during post monsoon season. Also the treated UWW showed a range from 520 mg/l during pre monsoon to 980 mg/l during post monsoon. TDS of untreated and treated UWW at all the three locations lie within slight to moderate range of the FAO irrigation water quality standards. The primary treatment brought certain reduction in EC and TDS. Muthukumaran *et al.* [9] also reports that primary treatment reduces the EC and TDS concentrations. However it is exceptional at the treatment plant at location No. 3, where there is increase in EC and TDS in the treated UWW, which is understandable because suspended materials along with the salts are not settled completely. Excessive total salt concentration or excessive levels of
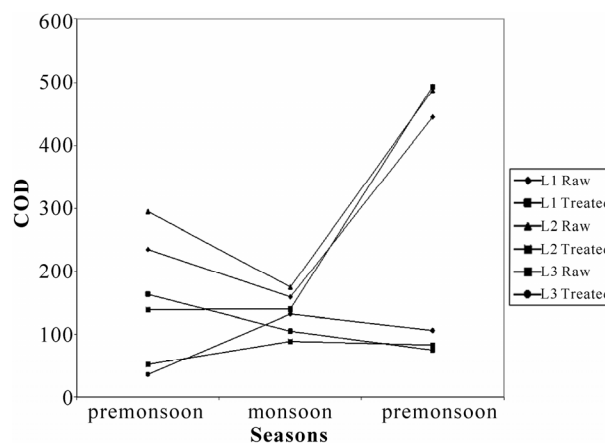

Figure 5. COD of treated and untreated UWW.

some potentially toxic elements can have detrimental effects on plant health and/or soil conditions. The EC and TDS of treated and untreated UWW is shown in **Figures 6** and **7** respectively.

3.4. Suspended Solids (SS)

The results of the present study show that in the untreated UWW, SS ranged from 40mg/l during premonsoon season to 360 mg/l highest during post monsoon season. SS in the treated UUW ranged from 20 mg/l during dry season to 60 mg/l during monsoon and post monsoon season. The primary treatment has considerably reduced the SS concentration. However the concentration of SS exceed the general standard of 20 mg/l stipulated by the State Pollution Control Board for discharge of effluent on land for irrigation.

3.5. Sodium Absorption Ratio (SAR)

SAR indicates the effect of relative cation concentration on Sodium accumulation in the soil. Present study shows SAR in untreated UWW from 1.08 to 4.76 during monsoon and premonsoon seasons respectively. Also in treated UWW, SAR ranged from 1.24 to 6.2. SAR in the untreated and treated waste water lies within the slight to moderate range of the FAO irrigation water quality standards. The SAR in the untreated and treated UWW is high during premonsoon season when compared to other seasons. There is no significant reduction in SAR in the treated UWW and SAR of the treated UWW at location No.3 is highest because of high concentration of sodium and incomplete treatment process. Continued use of water having SAR can lead to a breakdown in the physical structure of the soil. Any increase in the SAR in irrigation water increases the SAR of the soil solution, which ultimately increases the exchangeable sodium by the soil, leading to the loss of permeability. The SAR of untreated and treated UWW is shown in **Figure 8**.
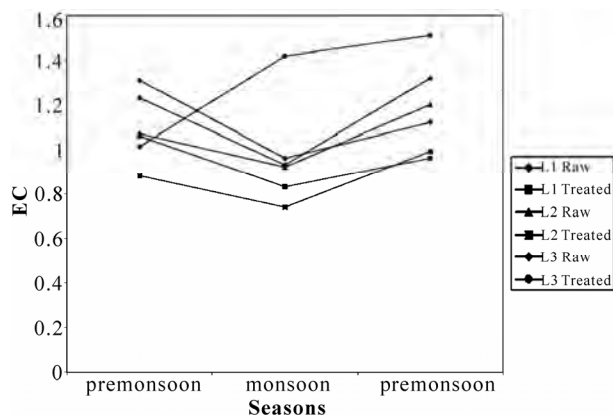
Figure 6. EC of treated and untreated UWW.
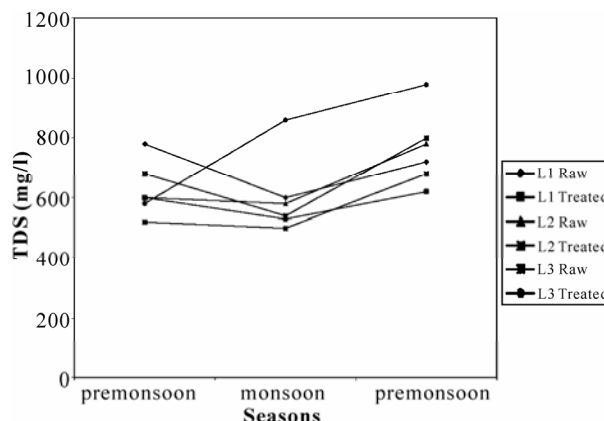
Figure 7. TDS of treated and untreated UWW.
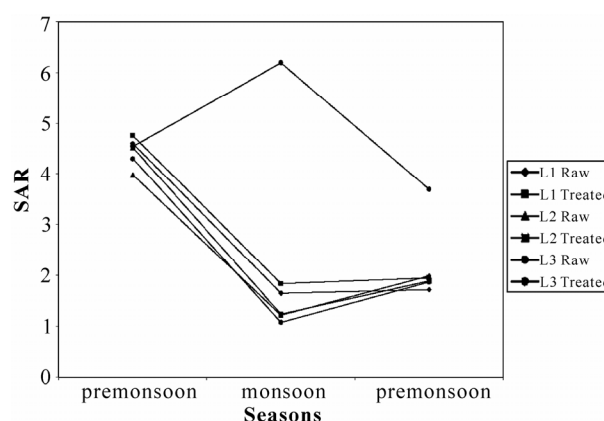
Figure 8. Sodium Absorption Ratio of treated and untreated UWW.

3.6. Nitrate (NO$_3$-N)

NO$_3$-N is a necessary primary macronutrient for plants that stimulates plant growth and is usually added as a fertilizer but can also be found in wastewater as nitrate, ammonia, organic nitrogen or nitrite [10]. The most important factor for plants is the total amount of nitrogen (N) regardless of whether it is in the form of nitrate-nitrogen (NO$_3$-N), ammonium nitrogen (NH$_4$-N) or organic-nitrogen (Org-N) but by reporting in the form of total nitrogen comparisons can be made [11]. The concentration of nitrogen required varies according to the crop with more sensitive crops being affected by nitrogen concentrations above 5 mg/l, whilst most other crops are relatively unaffected until nitrogen exceeds 30 mg/l.

NO$_3$-N results of untreated UWW show a range from 0.04 mg/l to 0.4 mg/l during dry seasons. In treated UWW, NO$_3$-N ranged from 0.07 mg/l to 0.32 mg/l during dry season. The NO$_3$-N of untreated UWW ranged from minimum of 0.04 mg/l during premonsoon to a maximum of 0.4 mg/l during post monsoon at location No. 1. The NO$_3$-N of treated UWW ranged from a minimum of 0.07 mg/l during post monsoon to a maxi-

mum of 0.61 mg/l during monsoon at treatment plant at location No. 2. Due to incomplete treatment process and the waste water becoming more enriched in nutrient, the concentration of the NO_3-N is increased in the treated UWW. However the values lie within the safe range of restriction on use of FAO irrigation water quality standards.

3.7. Phosphate (PO_4)

Phosphorus is also a primary macronutrient that is essential to the growth of plants and other biological organisms but quantities can be excessive and if the concentrations in water are too high noxious algal blooms can occur. Phosphates are classified as orthophosphates, polyphosphates and organic phosphates. Municipal waste waters may contain between 4 and 16 mg/l of phosphorus [5].

Wastewater with 6-20 mg/l phosphorous increases the productivity of the crops and when the concentration exceeds 20 mg/l the availability of copper, iron and zinc is reduced in alkaline soils [7]. The highest value of dissolved phosphate reported in the present study is 26.4 mg/l in the untreated UWW at location No.1 during pre monsoon season. The primary treatment brought some reduction in phosphate concentration, but it is exceptional in treatment plant at location No. 2 during pre-monsoon and post monsoon seasons.

Wastewater normally contains low amounts of phosphorous, so its use for irrigation is beneficial and does not negatively impact the environment. This is the case even when wastewater effluents with high concentration of phosphorous are applied over long periods of time although, because phosphorous builds up at the soil surface, it can affect surface waters through soil erosion and runoff [7]. The concentration of PO_4^{3-} of untreated and treated UWW is shown in **Figure 9**.

3.8. Potassium

Potassium is not an integral part of any major plant component but it does play a key role in a vast array of physiological processes vital to plant growth, from protein synthesis to maintenance of plant water balance. Potassium is a macro-nutrient that is present in high concentrations in soils but is not bio-available since it is bound to other compounds. Generally, wastewater contains low potassium concentrations insufficient to cover the plant's theoretical demand, and use of wastewater in agriculture does not normally cause negative environmental impacts [12].

Potassium may originate from human faeces and urine disposal, as human faeces has on average 1.6% and urine has 3.7% (dry weight) potassium. The concentration of potassium of untreated UWW was recorded highest of 46

mg/l during premonsoon season to a minimum of 5.1 during monsoon. The treated UWW show a maximum of 102 mg/l during post monsoon season and a minimum of 3 mg/l during monsoon at the treatment plant at location No. 3. The primary treatment has not significantly reduced the concentration of potassium at plant No. 1 and 2 during monsoon and post monsoon season which may be attributed to the incomplete treatment and may be due to dumping of the municipal solid waste at the outlet of treatment plant at location No. 2. The concentration of potassium of untreated and treated UWW is shown in **Figure 10**.

3.9. Heavy Metals Concentration

Trace quantities of many metals can be found in wastewaters, particularly industrial waste but also arising from domestic waste, for example from household cleaning products. Many of these metals are necessary for growth of biological life but only in trace concentrations; if the required concentrations are exceeded they can become toxic and thus interfere with the potential beneficial uses

Figure 9. Phosphate of treated and untreated UWW.
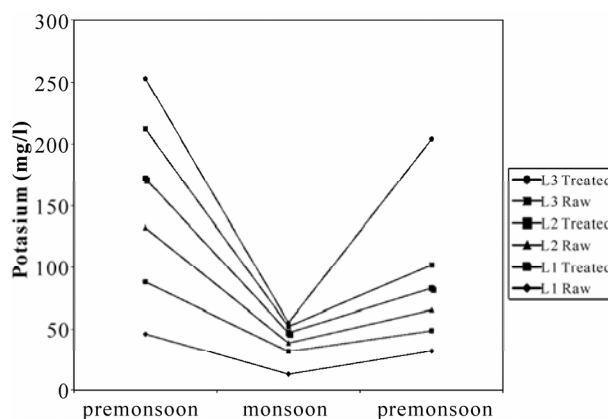
Figure 10. Potassium of treated and untreated UWW.
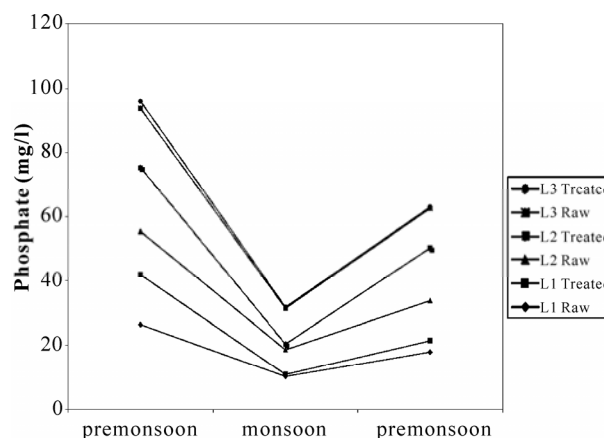
of wastewater.

The untreated and treated UWW were analyzed for identification of metals which were either beneficial for plant growth, or likely to cause damage to crops or impact on human health. Heavy metals like Nickel, Copper, Cadmium, Chromium, Iron, Zinc and Lead were analyzed using Inductively Coupled Plasma atomic emission Spectroscopy techniques (ICP-AES). Among these Iron and Copper can help plant growth and development, but other heavy metals directly or indirectly affect the plant growth and development to various degrees and above different threshold levels. The results are shown in **Table 2**.

The results of heavy metal concentration of UWW showed that lead was not detected in any of the samples. Concentration of Iron in untreated UWW at all locations and during all seasons is on the higher side when compared in the treated UWW. Comparatively iron concentration in untreated and treated UWW is highest during post monsoon season. This might be due to the combine sewer system receiving arising runoff during the wet season which contains Iron, one of the most abundant elements in the environment [13] and also it may be due to corrosion of water supplying pipes.

Concentration of Copper, nickel, and Zinc in untreated UWW at all locations and at all seasons is more than in treated UWW. Whereas, the concentration of Cadmium and chromium in untreated and treated UWW does not significantly show the difference. Similar reports are recorded by [14]. Among the heavy metals, the concentration of Chromium, Cadmium, Nickel, and Iron (in post monsoon season) are exceeding the FAO standards. Also it can be implied that biological treatment process which mainly established for the removal of organic matters and nutrients can additionally remove metal contents from wastewater

4. Conclusions

The parameters like BOD and COD of untreated UWW exceed the CPCB permissible limit for disposing the UWW on land for irrigation, but EC, TDS and SAR are within the average range of the FAO irrigation water quality standards. The undesirable effects include that the entire quantity of urban waste water generated at Mysore city limits is not collected for treatment and as such the untreated waste water finds its way into water bodies and also to the agricultural land thus resulting in contamination of water bodies and soil pollution.

Seasonal variations act upon a number of factors that influence the toxicity of UWW and its effects in the receiving environment. The factors affected include dissolved oxygen concentrations in receiving waters, temperature of the wastewater and the receiving environment,

water levels and assimilative capacity, the types of contaminants that accumulate on urban surfaces and the efficiency of UWW treatment plants.

Nutrients are clearly present in the wastewater, and especially phosphorus is high in content. The presence of nutrients can be beneficial for agricultural use, but the quantities need to be known before they are applied. In order to bring down the deterioration of water bodies and soil condition, it is recommended Best Management Practices (BMPs) by connecting all the missing links to the waste water treatment plants and upgradation and continuous operation of the existing treatment plants. Solid waste has to be prevented from being dumped into the canals and near the waste water treatment plants by ensuring regular collections, increasing composting and recycling in collaboration with the existing solid waste management programs in the area.

5. Acknowledgements

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Scientific
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Impact of Water Resources Protection on Local Ground Water Market

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Abstract

Tanks are important means to conserve water resources in semi-arid areas. Tank irrigation in general, and in Tamil Nadu in particular, has a long history, and it can be traced to prehistoric times. Poor management of the integrated tank system including its structures and distribution system is one of the major reasons for decline of tank irrigated area. Rehabilitating tanks enhanced groundwater recharge leads to increased water table in the wells located in the command area. Hence well owning farmers can able to cultivate three times paddy crop in a year with the available ground water. Also they can provide water for adjacent non-well owning farmers for their second crop (during maturity stage) and third crop (whole season) cultivation by charging either cash or kind. This local ground water market helps villagers to cultivate more area and season, leads to efficient water use and increased income generation for the villagers. To prove the above statement a study was carried out in rural village of South India with an interview schedule and the data were analysed using SPSS (Statistical Package for Social Science). The result shows that the tank rehabilitation is highly correlated with local ground water market in post rehabilitation period.

Keywords: Agriculture, Groundwater Market, Tank Rehabilitation, Irrigation

1. Introduction

Localized village-level informal arrangement through which well owners sell irrigation services to other members of the community. Water may be lifted from open wells or tube wells, deep or shallow wells and may be transported to the buyers field either through lined or unlined field channels or through underground pipeline networks. According to [1], the emergence of water markets, both ground and surface water, has helped in increasing agricultural output. It has also widened the equalities in the rural areas and protected the interests of the small and marginal farmers and other weaker sections of the society. Where land holding are fragmented, most sellers of water are also buyers themselves. In areas irrigated through water markets, the intensity of irrigation may vary greatly. However, since the buyers can get water when they need, the productivity of water is high. Opportunity to buy small quantities of water at crucial periods of moisture stress enables small holders to take an additional crop. Water selling by private owners can have remarkable beneficial impacts on the incomes of

water buyers and the economy of the community as a whole [2,3]. Some of the findings from [4] study in eastern and western Utter Pradesh about trends in ground water irrigation indicate that significant changes have taken place in respect of socio-economic features, role of ground water irrigation including ground water markets, productivity of crops and changing face of the rural elite. Marginal farmers are the biggest beneficiaries of ground water market. Nearly 60% of the farmers of this category irrigated their crop by water purchased from well owners making ground water accessible to socially and economically deprived sections of the society [5] Efficiency and equity benefits of ground water markets have an inadequate empirical grounding and are based on a 'one size fits all' model of water market that is insensitive to how ground water markets are shaped by natural, social and historical factors [6]. Performance of water market is in terms of the quality of irrigation service available for the depth and breadth of water trade, and the presence or absence of monopoly pricing. The impact of water market on cropping intensity, cropping pattern, labour use and crop yields achieved by water buyer compared to water extraction mechanism owners. When water is sold

for cash, it may be priced on the basis of hectares (ha) irrigated of a particular crop or on the basis of hours of pumping [7].

Water markets emerging under the flat price power enable small farmers to gain access to water supplies at less than the full cost of pumping and infrastructure [8]. Due to operation on localised water markets, substantial proportion of the total benefit generated by private investment in water extraction mechanisms accrue to the resource poor non-water extraction mechanism owning farmers and even the landless. This proportion tends to increase as the water markets become more 'efficient' and the gap between the incremental pumping costs and water prices declines [9]. Ground water irrigation is a major source of livelihood not only for well owners, but also for water buyer and farm labourers [10].

2. Materials and Methods

This study was carried out in a rural village named 'Pelasur' of Thiruvannamalai district of Tamil Nadu, India. This tank was rehabilitated in the year 2001-2002 with World Bank funding of Rs. 40.01 lakhs. Twenty percent households of women and men farmers who owned atleast one irrigated plot of land under the 'Pelasur' tank command area were selected as respondents using Stratified sampling method. In order to achieve the proposed objectives, combination of quantitative and qualitative methods were used to gather information about local ground water market in pre and post rehabilitation period. Year of consideration for data collection were June 1998-May 1999 and June 2004-May 2005 for before and after rehabilitation respectively. The data thus obtained were first master tabled using appropriate coding and the variables for inclusion in analysis were selected and

transmitted into raw data files. Further, it was arranged into SPSS-files for statistical analysis which in fact proved to be a cumbersome part of the research. Since this study concentrates on tank and well water users both before and after rehabilitation, comparative analysis in SPSS was attempted using paired-samples t-test, which is a compare means analysis.

3. Results and Discussions

Table 1 elucidates the prevalence of water marketing in different seasons and stages of crop growth in 'Pelasur'. Irrespective of the crop cultivation season, the general scenario of buying and selling water is practised in this village. However, it is evident from the data that during second season, total number of ground water buying and selling is increased in post rehabilitation period. But buying and selling is not done by the same respondents in pre and post rehabilitation period. With the help of tank water, farmers cultivate entire first season paddy crop and for second season they are depending on neighbouring well owning farmers. When tank water fails to full fill the needs, especially during flowering and maturity stages of paddy crop, some 4 to 5 watering from well will save crop from reduction in yield. It is estimated that out of 60 bags/ha (each bag weights about 75 kg) only half the quantity will be received due to the above situation (*i.e.* 30 bags). Income realised through these 30 bags of paddy/ha is Rs. 15,000/-, which is considered to be very low. Hence, input and out put costs per ha are the same and there will not be any profit as claimed by the respondents. So, non-well owning farmers are ready to buy water from well owning farmers in order to avoid crop losses.

Mode of payments as claimed by well owners is either

Table 1. Season wise ground water buying and selling before and after rehabilitation by number of Pelasur respondents.

S. No.	Crop growth stage	Number of Respondents Ground water buying						Number of Respondent Ground water selling						A	B
		Second season		Third season		Annual		Second season		Third season		Annual			
		BR	AR	BR	AR	BR	AR	BR	AR	BR	AR	BR	AR		
1	Land preparation and Nursery	3	4	0	11	0	0	4	9	1	12	0	5	**8**	**41**
2	Flowering	0	7	0	0	0	1	2	5	0	0	0	6	**2**	**19**
3	Maturity	0	0	0	0	0	1	1	12	0	0	0	8	**1**	**21**
4	Whole season	0	6	0	0	0	0	6	0	3	7	0	6	**9**	**19**
5	**Total**	**3**	**17**	**0**	**11**	**0**	**2**	**13**	**26**	**4**	**19**	**0**	**25**	**20**	**100**

Note: BR-Before rehabilitation; AR-Before rehabilitation; A-Total number of respondents involved in ground water market before rehabilitation; B-Total number of respondents involved in ground water market after rehabilitation.

in cash or in kind. In case of cash, it is decided on the basis of ha/hr of irrigation. It is Rs. 75/ha/watering (each watering 6 hr/ha) for paddy crop. The amount normally earned by sellers is approximately Rs. 2,250/ha/season. In case of kind, the payment is one third of the yield/ha/season. Hence, 20 bags will be given to the water sellers. Then amount earned by sellers is Rs. 10,000/ha (each bag of paddy costs Rs. 500/-). The latter one is beneficial for the sellers, and so they make use of the buyer's critical situation and earn Rs. 10,000/ha just through selling their ground water. Local water market is not practised for annual crop since only well owning farmers are involved in sugarcane cultivation and they are satisfied with their own well yield. But still very few farmers owning wells with poor yield are the reliant of neighbouring wells in the last 3 months of crop growth. During this three months period, the need may be of 6 watering (15 hr/watering/ha) to achieve a normal crop yield of 100 tons/ha. So, the buyer has to pay Rs. 6,300/ha (Rs. 70/- to purchase water for 90 hours). Payment in cash to buy water for sugarcane is the common practice in Pelasur. Availability of water resources, scale and quality of adoption of irrigated farming technologies, progress of rural electrification, quality of power supply and extent of land fragmentation are among the factors that seem to influence the pace of development of water market [2].

Few well owning farmers, who are involved in agriculture as well as working in the Government and private sectors, leave their land fallow especially during the summer season. However, they sell water for needy farmers of adjacent non-well owning farmers. Crop sharing contract system is prevailing in this village where the seller provides only water while the buyer provides land, labour, manure and other inputs and both of them share the crop yield. One interesting case seen in Pelasur is that few respondents cultivating sugarcane alone sell water to nearby non-well owning paddy cultivators and get back an average of 10 bags/ha/year for their household consumption. Each bag of 75 kg paddy gives 40 kg of rice. Hence, the total 400 kg of rice is sufficient for the whole year for a household comprise 6 members. Only one respondent owning diesel pump is selling water to his adjacent field for second season paddy crop during its maturity stage. It is informed that buyers pay Rs. 50/hr/ha and also arrange for the diesel. Hence, for 4 to 5 watering, the diesel pumpset owners can earn Rs. 2,500/ha/season. Even though the local water market is successfully carried out in Pelasur for increasing overall productivity, sometimes farmers will not charge for their relatives because it is a homogenous caste group village. The intensity of irrigation may vary greatly in areas irrigated through water markets. However, the productivity of water is high since the buyers can get water when they need. Opportunity to buy minimum quantities of water at crucial stages of moisture stress enables small holders to

take an additional crop. Water selling by adjacent well owners can have remarkable beneficial impacts on the income of water buyers and the economy of the community as a whole [2].

The output confirms the result that a significant positive relationship exists between local ground water market buying and tank rehabilitation ($r = 0.453$, $p < 0.05$). Therefore local ground water market buying is associated with tank rehabilitation but less correlated. Minimum ground water market buying for annual crop is the reason behind least correlation. The observations related to local ground water market buying and tank rehabilitation, which were randomly assigned to ensure that responses are due to the tank rehabilitation and not due to other factors. Paired t-test technique was used to test the null hypothesis and compare the results to prove that there is a difference between local ground water market buying before and after tank rehabilitation. The analysis output reveals that there is a difference between local ground water market buying by the respondents in pre and post rehabilitation periods. Hence, it is concluded that tank rehabilitation significantly improves the local ground water market buying, $t (101) = 3.154$, $p < 0.05$. The output confirms the result that a significant positive relationship exists between local ground water market selling and tank rehabilitation ($r = 0.905$, $p < 0.05$). Therefore local ground water market buying is associated with tank rehabilitation but highly correlated. Increased ground water market selling for annual crop is the reason behind high correlation.

The observations related to local ground water market selling and tank rehabilitation, which were randomly assigned to ensure that responses are due to the tank rehabilitation and not due to other factors. Paired t-test technique was used to test the null hypothesis and compare the results to prove that there is a difference between local ground water market selling before and after tank rehabilitation. The analysis output reveals that there is a difference between local ground water market selling by the respondents in pre and post rehabilitation periods. Tank rehabilitation significantly improves the local ground water market selling. Hence, it is concluded that tank rehabilitation significantly improves the local ground water market selling, $t (101) = 2.282$, $p < 0.05$.

4. Conclusions

Village tanks occupy a significant position in irrigation and in the local ecosystem in low rainfall areas. They have been one of the most important resources for the rural communities in the Indian subcontinent where there is no perennial river of importance. The performance of tanks is coming down due to various reasons. Since the tank irrigated areas benefit the marginal cultivators the livelihood of these communities gets affected. Conserva-

tion and the revival of local management of these tanks is therefore the urgent need of the hour to help the poor communities to come out of their deprivation and have a secure livelihood. Hence the better ground water recharge through tank rehabilitation helps both well owning farmers and non-well owning farmers (marginal farmers) for income generation through increased cultivated area and number of season crop cultivated. Therefore tank rehabilitation is associated with local ground water market buying and selling.

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Kinetic Studies for an Aerobic Packed Bed Biofilm Reactor for Treatment of Organic Wastewater with and without Phenol

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Abstract

A laboratory scale aerobic fixed film bioreactor packed with glass beads for biofilm growth was used to evaluate the removal efficiencies of COD and phenol for a carbohydrate—phenol mixture in wastewater. It was done by an indigenous mixed culture inoculums developed after collecting sludge from a return line of an activated sludge plant. The test result on continuous flow in the above biofilm reactor indicated an optimum hydraulic loading range of 4-6.4 $m^3day^{-1}m^{-2}$ for attainment of reasonable amount of COD removal in case of carbohydrate substrate only. The COD removal efficiency, however, gradually depleted from 100% to 54% by gradual increase in organic loading (OLR) from 0.72-4.32 $kgday^{-1}m^{-3}$, beyond which removal was not significant. For the identical loading conditions, in presence of phenol in the substrate along with carbohydrate, the COD removal was observed varying from 100-40% in the above organic loading range. The COD removal kinetics in presence of phenol also shows a decreasing trend compared to data obtained without the presence of phenol in wastewater that reveals biological inhibition. The experimental data were fitted in a simple plug flow model for evaluating the zero order, first order and Monod form of rate equations to evaluate the kinetics. It was found that Monod type rate equations combining a zero and first order rate expression is the best fit for the above hydraulic and organic loading that gives a best fit half velocity constant value of 35 mgL^{-1} ($R^2 = 0.9612$).

Keywords: Packed Bed Reactor, Biofilm, Mixed Culture, COD Removal, Phenol, Kinetic Model

1. Introduction

Biofilm reactor is a popular method for biological treatment of wastewater to combat high organic strengths owing to enhanced mean cell residence time and economical oxygen supply [1-4]. However, adequate mixing in the biofilm reactor is important to ensure uniform distribution of substrate, sufficient contact between the microorganism and substrate and prevention of localized accumulation of toxic matter. In absence of recirculation, mixing can be achieved to some extent by uniform supply and distribution of oxygen in the reactor. Wastewater emanated from petrochemical, pharmaceutical, coke oven plant etc contains high phenolic compounds along with appreciable amount of COD. COD removal from wastewater can be obtained by either pure culture organism or by mixed culture system. As wastewater from

industry contains mixed nature of organic matters and toxic compounds, and the maintenance of pure culture in industry for the treatment process of wastewater is difficult to achieve, so it is advisable to use mixed culture for treatment of wastewater having high COD and phenol concentration.

Phenol biodegradation by pure cultures of bacteria has been described by substrate inhibition models [4-7]. Otherwise, when mixed cultures are used there is no unique kinetic model for general agreement, but phenol is often considered as inhibitory at high concentrations [8-10]. At low substrate concentration, however, the inhibition is negligible; the model proposed by Monod may be used to describe the biodegradation process by either pure or mixed cultures. Furthermore, if the substrate concentration is much lower than the half-velocity constant (K), then a first-order kinetic model often applies.

Wastewater having high concentration of COD and phenolic compounds can be treated mainly by either physico-chemical method e.g. adsorption, chemical co-agulation, membrane filtration process or by biological methods such as activated sludge or anaerobic cultures under acclimatized condition. Some researchers consider activated sludge more attractive due to its various ad-vantages [11-13]. Activated sludge reactors have been widely used for COD and phenol removal from industrial wastewater [14-16], but immobilized cell reactors offer several advantages over suspended cell reactors. These include higher biomass concentrations, allowing higher loading rates, and resistance to shock loading of inhibi-tory compounds, then requiring less time to revert to normal operation. Fixed film reactors are receiving in-creasing interest in wastewater treatment. Most of the works on fixed bed reactor have been done in anaerobic condition [17-19]. Few works have been done on com-bined aerobic-anaerobic fixed film reactor system for COD removal. G. R. Moosavi *et al.* [20] investigated the COD removal for high strength organic wastewater in this reactor and found COD removal efficiency about 95% under organic loading of 0.8-7.6 kg CODm^{-3}day^{-1}. R. Del Pozo and V. Diez [21] have studied the COD re-moval for organic matter containing wastewater in aero-bic-anaerobic packed bed reactor and they found the ef-ficiency to be 92% at organic loading of 0.39 kg COD m^{-3}day^{-1}. They have also found that most COD removal occurred mainly by aerobic process. In another study, Lorenzo Bertin *et al.* [22] has shown that an aerobic re-actor, fed with olive mill effluent from anaerobic GAC reactor, could be a stable process to remove 24% and 39% of organic load under 50.42 and 2.04 gL^{-1}day^{-1} of COD and phenol loading rates, respectively. In the pre-sent work, the activated sludge was collected from a re-turn line of a nearby sewage treatment plant. The micro-bial consortia was grown on glass beads forming a fixed biofilm and used for further experiment. The main pur-poses of the present investigation are as follows: 1) To understand the behavior of the reactor under different operating conditions like different hydraulic and organic loading and finding out the optimum operating condi-tions of it. 2) To find out the change in efficiency of the reactor to treat the carbohydrate containing wastewater in the presence and absence of phenol. 3) To use the ex-perimental data for fitting in the kinetic model for COD removal.

2. Materials and Methods

2.1. Collection of Inoculum

Biomass from a return line of a sewage treatment plant operating as activated sludge process was collected as inoculum for the reactor starting up. The inoculum was first grown as batch mode with nutrient medium in the reactor and air was supplied from the bottom through a mini-compressor. The culture was replenished with fresh substrate medium time to time so that the bacterial cul-ture can get sufficient nutrient for their growth. After 6 weeks of operation, a thin film coating was found to de-velop on the glass beads, and then the operation was changed to continuous mode. Microorganisms were first grown on easily degradable substrate (up to 1000 mgL^{-1} COD contributed) and then acclimatized to phenol. Ac-climatization of the activated sludge sample grown in the trickle bed reactor was carried out to initiate the ability of the sludge to biodegrade phenol along with other or-ganic matter.

2.2. Nutrient Medium and Culture Condition

The required nutrients for the growth of the microorgan-isms were supplied by the medium with compositions as follows in 1L of solution: Glucose 0.5 g, peptone 0.2 g, beef extract 0.2 g, Yeast extract 0.2 g, K$_2$HPO$_4$ 0.3 g, KH$_2$PO$_4$ 0.15 g, NH$_4$Cl 0.2 g, CaCl$_2$ 0.1 g, FeCl$_3$ 0.1 g, MgCl$_2$ 0.1 g. The glucose concentration was varied to vary the inlet COD to the reactor according to need of organic loading variation, while peptone and yeast ex-tract concentration dropped to zero during the study of COD removal performance of the reactor.

2.3. Analytical Procedure

COD was measured by standard closed reflux method in HACH (USA) make reflux apparatus. The sample was oxidized with K$_2$Cr$_2$O$_7$ in a strong acidic condition (H$_2$SO$_4$), followed by titration of the excess dichromate with Mohr salt solution. Phenol was determined by stan-dard 4-amino antipyrene method.

2.4. Experimental Set Up

The experimental set up is shown in **Figure 1**. The bio-reactor was 65 cm high with 2.5 cm internal diameter of cylindrical Borosil make glass column. Glass beads with average 3 mm diameter were used as inert support media for the biofilm growth. There was an in-built perforated support at the bottom to hold the glass beads. The treated water after filtration was taken as the effluent. Inlet and outlet for the wastewater were provided at the top and bottom of the bioreactor, respectively. There were two other sampling ports at 25 cm and 45 cm from the top of the reactor for collecting intermediate samples at times. The synthetic wastewater was prepared with glucose, as concentration ranging from 100-400 mgL^{-1} by diluting the glucose stock in distilled water along with necessary nutrients in proportionate amount as stated above. The sy-nthetic wastewater was fed to the reactor from an aspirator
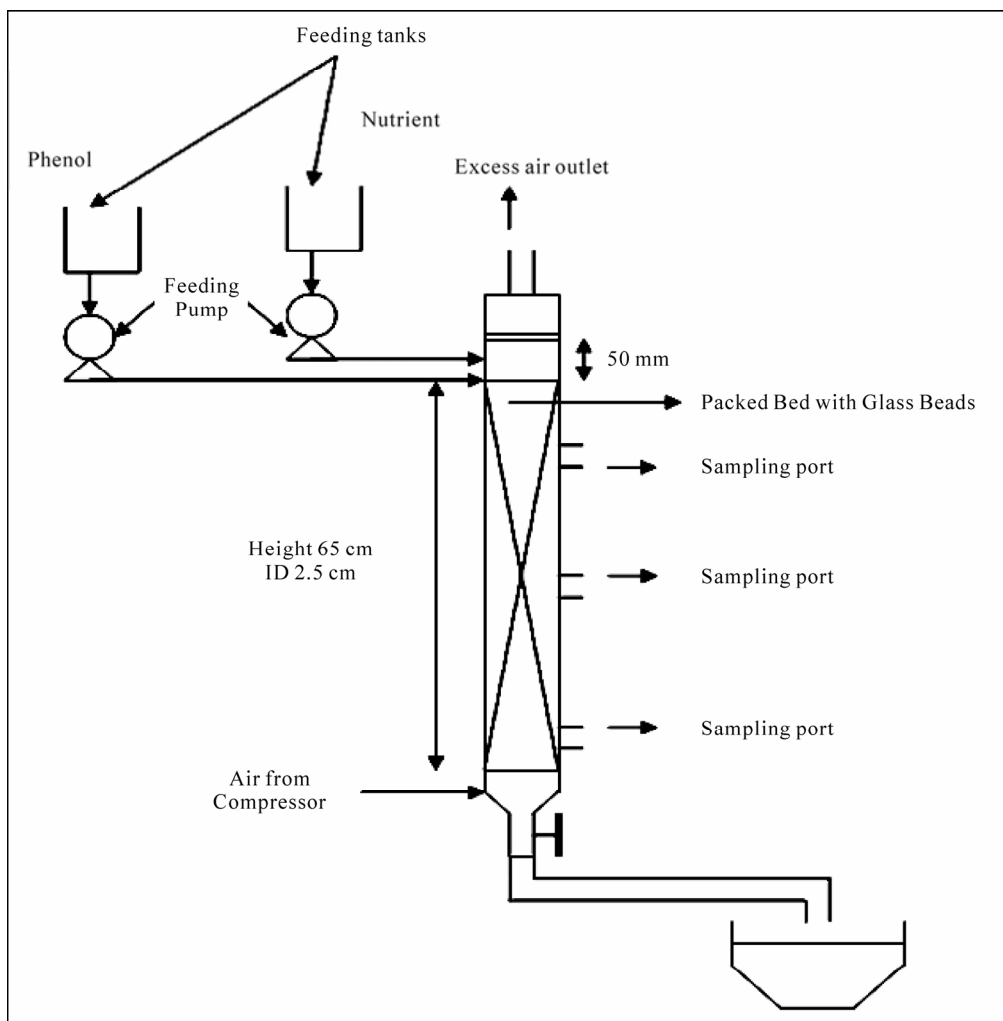
Figure 1. Experimental set up.

bottle in the down-flow mode. The effluent was collected from the bottom outlet of the reactor and filtered to remove the suspended solids. Then the samples are tested for residual COD. All the COD removal studies were done for a single pass of wastewater though the bioreactor without any recirculation. After finding out the optimum hydraulic and organic loading rates for the reactor, 50 mgL^{-1} phenol was fed to the reactor having glucose as the major carbon source other than phenol in the synthetic wastewater. In these set of experiments, residual COD was tested in the effluent.

2.5. Hydraulic Loading Rates (HLR)

The flow rates of the wastewater fed to the reactor were calculated in order to operate at low hydraulic loading rates. Although there is no general agreement about limits for operating in this regime, but first superficial velocities were selected in such a way that the corresponding hydraulic loadings ranged from 4.27-9.96 m^{3}m^{-2}day^{-1}.

The reactor performances in steady state conditions were evaluated at four hydraulic loading rates: 4.27, 6.4, 7.11 and 9.96 m^{3}m^{-2}day^{-1}.

2.6. Organic Loading Rates (OLR)

For each of the hydraulic loading rates, initially, the bioreactor was fed with synthetic wastewater containing carbohydrate without phenol at different organic loadings. Total COD of the influent for each run is measured. The inlet COD concentrations range were 100-400 mgL^{-1} corresponding to organic loading rate varying from 0.72-4.8 Kgm^{-3}day^{-1}. Percentage removal of COD at each HLR and OLR were determined. The COD removal performances of the reactor at the optimum HLR were also examined for treatment of wastewater having 50 mgL^{-1} of phenol along with glucose as organic matter. The concentration of glucose in the influent wastewater was varied in such a way that the total organic loading fell within the range of OLR stated above even in presence

of phenol in inlet wastewater.

3. Results and Discussion

3.1. Effect of Hydraulic Loading Rates on Organic Removal Efficiency

Figure 2 shows that the percentage COD removal vs. hydraulic loading at different organic loadings. It is seen from the above figure that for a particular HLR, the removal efficiency decreases as OLR increases. Organic matter was well degraded almost 100% corresponding to the hydraulic loading of 4.27 $m^3m^{-2}day^{-1}$ and organic loading of 0.72-1.44 $Kgm^{-3}day^{-1}$. At OLR of 2.037 Kg $m^{-3}day^{-1}$, the percentage COD removal was 88.33, but a further increase in OLR, a sharp descend of COD removal to 60% is observed for the HLR at 4.27 $m^3m^{-2}day^{-1}$. Further, when HLR is increased to 6.4 $m^3m^{-2}day^{-1}$, then also 100% COD removal is achieved at OLR of 1.08 Kg $m^{-3}day^{-1}$. The COD removal was decreased to 83% when the applied OLR was doubled at 2.16 $Kgm^{-3}day^{-1}$, keeping the HLR constant at 6.4 $m^3m^{-2}day^{-1}$. While studying the performance of the reactor at higher HLR *i.e.* at 7.11 $m^3m^{-2}day^{-1}$, 100% removal was possible to achieve only up to an OLR of 1.2 $Kgm^{-3}day^{-1}$, beyond that, a further increase in OLR to 2.4 $Kgm^{-3}day^{-1}$, the percent removal was declined significantly to 66.5%. An Increase of HLR up to a value of 9.96 $m^3m^{-2}day^{-1}$ exhibited poor removal efficiency.

3.2. Optimum Operating Range of Bioreactor

From the reactor performance data as given in **Table 1**, and shown in **Figure 2**, it is evident that the optimum
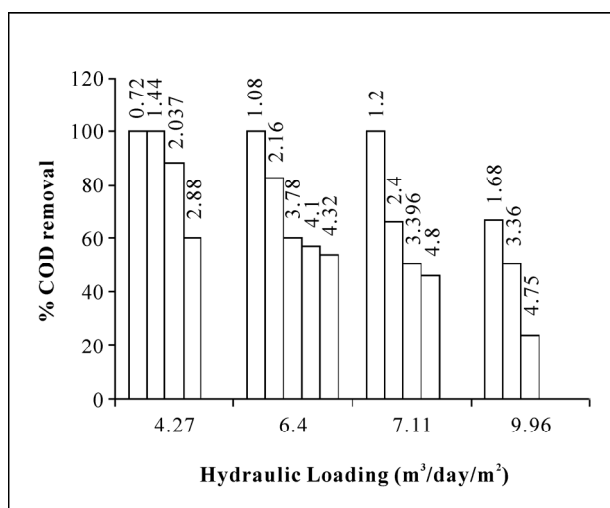
Figure 2. Percentage COD removal from wastewater containing organics (no phenol) versus hydraulic loading at different organic loading (noted at the top of each bar).

Table 1. Rector performance a different HLR an OLR with and without phenol condition.

Hydraulic loading (HLR) $m^3m^{-2}day^{-1}$	Organic Loading (OLR) $Kgm^{-3}day^{-1}$	%R[a]	%R[b]
9.96	1.68	67	-
9.96	3.36	50	-
9.96	4.75	24	-
7.11	1.2	100	-
7.11	2.4	66.5	-
7.11	3.396	50	-
7.11	4.8	46	-
7.11	4.2	50	-
6.4	4.1	57	-
6.4	4.32	54	40
6.4	3.78	60	-
6.4	3.24	-	50
6.4	2.16	83	55
6.4	1.08	100	92
4.27	0.72	100	100
4.27	1.44	100	91.5
4.27	2.037	88.33	-
4.27	2.16	-	66
4.27	2.88	60	50

%R[a]: Percentage removal of COD without phenol
%R[b]: Percentage removal of COD with 50 mg/L phenol

range of HLR for the bioreactor should be between 4-6.4 $m^3m^{-2}day^{-1}$ and the optimum OLR up to 4.32 $Kgm^{-3}day^{-1}$. It was also observed that the COD removal efficiency shows a range from 100-54% at the above corresponding HLR range for the organic loading 0.72-4.32 $Kgm^{-3}day^{-1}$ as shown in **Figure 2**. G. R. Moosavi *et al.* [20] have tested an upflow anaerobic/aerobic packed bed reactor for COD removal studies. They have operated their reactor with volumetic flow rate of 1.26 $Lday^{-1}$ and under organic loading rates of 0.8-7.6 $Kgm^{-3}day^{-1}$. They have found that under the tested organic loading rates, the aerobic section of the reactor could remove COD in the range of 38-92%. It was also shown by their study that 92% COD removal was achieved at organic loading rate of 4.7 Kg $m^{-3}day^{-1}$. Their result of COD removal agrees well with our present experimental studies under similar range of operating condition.

3.3. Effect of Phenol on Organic Removal Rate

Phenol was found to possess a marginal inhibitory effect on the organic matter biodegradation in the reactor corresponding to a concentration of 50 mgL^{-1} of phenol. From **Figure 3** and **Figure 4** it is evident that in presence of phenol, the total COD removal efficiency showed a decreasing trend than treating the wastewater without phenol. The percentage removal of COD ranged from 100-54%, in case of without phenol in the inlet wastewater. The range was marginally fallen to 100-40% in presence of phenol along with other organic substrates at optimum hydraulic and organic loading range and under same operational conditions as stated above. The comparative performance was listed in **Table 1**. Studies have been done by Lorenzo Bertin *et al.* [22] for real life olive mill effluent. They have found that post treatment of that effluent (coming out from reactor packed with granular activated carbon) by aerobic packed bed reactor showed only 24% and 39% COD and phenol removal respectively, when charged the reactor with 50.42 ± 4.78 gL^{-1} day^{-1} of COD loading and 2.04 ± 0.27 gL^{-1}day^{-1} of phenol loading rate. Here percentage COD removal is far less compared to the result obtained in present study. This is because of the fact that both COD and phenol concentrations were sufficiently high in olive mill effluent than that tested in our study.

3.4. Kinetic Model

A simple kinetic model to represent the biodegradation behavior obtained in the laboratory was used to understand the reaction rate. The major independent variables in the process were the flow rate (Q), inlet concentration of organic matter in terms of COD (Cin), Outlet COD concentration of the stream (Cout). Assuming there was no growth during the experiment and no diffusion resistance within the biofilm, the usual plug flow model could be used as proposed by [23],

$$V = \frac{Q}{(1-e)a}\int_{Cin}^{Cout}\frac{dC}{-r} \qquad (1)$$

where *V* is the packing bed volume, "*a*" is the dry weight of biomass per volume of packing, "*e*" is the void fraction, and apparent reaction rate term *r* can be defined as $r = $ COD removed time^{-1} biomass^{-1}.

Assuming zero order kinetics, where $r = k$, k is a constant and *r* is independent of *C*, then Equation (1) can be easily integrated to give a well known linear relationship between Cin and Cout with slope 1.00 as follows:

$$QCout = QCin - (1-e)kVa \qquad (2)$$

The experimental data were plotted assuming zero order kinetics in **Figure 5**, showing not suitable for present
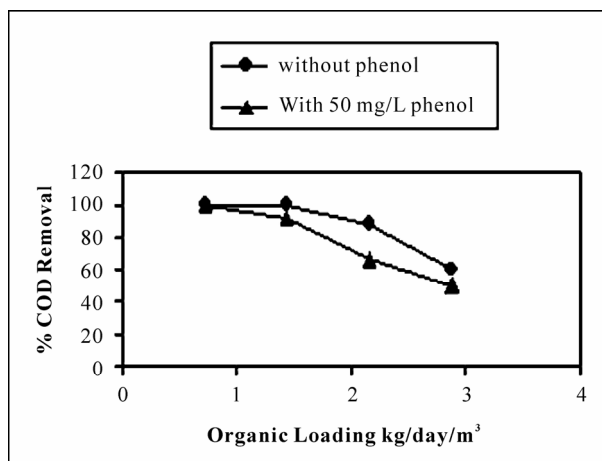
Figure 3. Comparison of percentage COD removal from wastewater containing only carbohydrate (without phenol) and carbohydrate in presence of phenol, against different organic loadings at 4.27 m³day⁻¹m⁻² hydraulic loading rate.
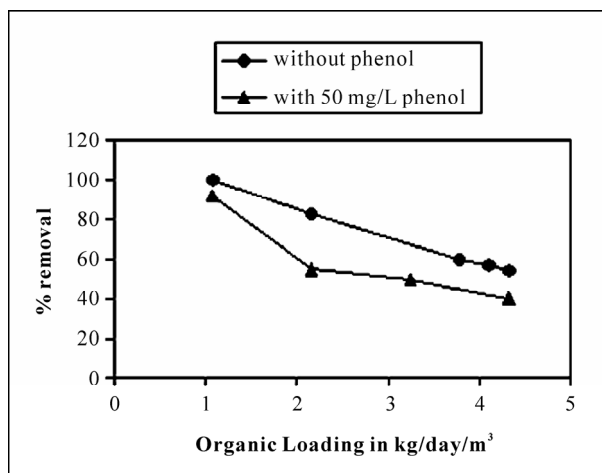
Figure 4. Comparison of percentage COD removal from wastewater containing only carbohydrate (without phenol) and carbohydrate in presence of phenol, against different organic loadings at 6.4 m³day⁻¹m⁻² hydraulic loading rate.

experiment as slope of the straight line far away from 1.00.

If the fist order kinetics is assumed where, $r = kC$, then we obtain,

$$V = \frac{-Q}{(1-e)ka}\left[\ln\frac{Cout}{Cin}\right] \qquad (3)$$

This can be written as

$$Q\ln Cout = Q\ln Cin - (1-e)kaV \qquad (4)$$

Here a linear relationship between *Q* ln Cin and *Q* ln Cout is expected. Assuming first order kinetics, experimental data plotted in **Figure 6**. But the experimental data were not fitted well to this model following a R^2 value of 0.7779.
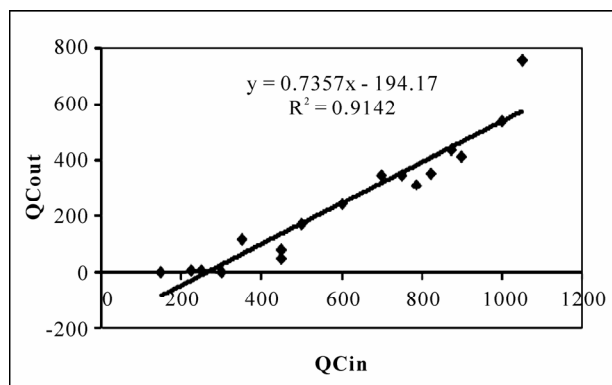
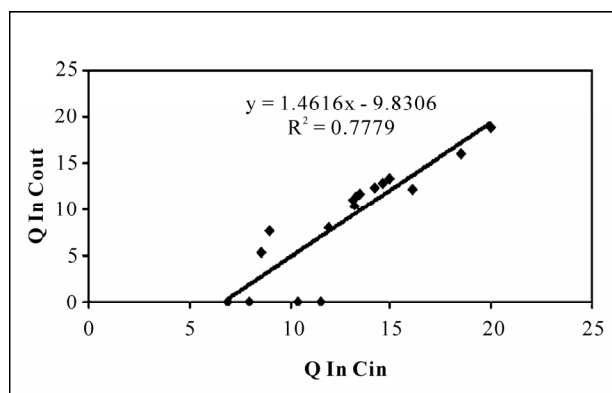**Figure 5. Zero order model for the bioreactor performance.**

Figure 6. First order model for the bioreactor performance.

However, the Monod form of rate equation combining the zero order and first order rate expression has been chosen, which seems to be more powerful and realistic expression for removal kinetics has the form

$$r = \frac{kC}{K+C}$$

where k is reaction rate constant with unit (time^{-1}. concentration^{-1}) and K is the half saturation constant having the same unit of COD concentration.

Putting this expression to Equation (1) we get,

$$V = \frac{-Q}{(1-e)ka}\left[(Cout - Cin) + K\ln\frac{Cout}{Cin}\right] \quad (5)$$

This can be rewritten as

$$Q(Cout + K\ln Cout) = Q(Cin + K\ln Cin) \\ -(1-e)kaV \quad (6)$$

In this form, the data can be linearized for a suitable K and plotting each Q (C + K ln C) term on linear paper. The best fit value of K is that gives best fit straight line (by maximizing the coefficient of determination R^2). Clearly the slope of the straight line plotted as Q (Cout + KlnCout) vs. Q (Cin + Kln Cin) is expected to be unity and intercept to be negative.

The same experimental data were tested for Equation (6) assuming different K values. Different K values and corresponding values of slope and coefficient of determination are listed in **Table 2**. Comparing the goodness of fit of the present data in Equation (6) with different K values, it was established that the rate equation described in Monod type form is most suitable for the present study. The half velocity constant values (K) are found between 25-35 mgL^{-1} for satisfying both of the conditions of values of slope and high value of R^2 (**Figure 7**, **Table 2**). But the study done by Tomás Wilson et al [24] shown that phenol biodegradation by mixed culture had followed zero order kinetics at 50 mgL^{-1} phenol concentration under batch mode. The difference in order of kinetics of that result with the present study may be due to the fact that they have studied the phenol degradation under high salt concentration (10% W/V NaCl) and by halophilic bacteria. But the culture for the present study was collected from activated sludge unit of coke oven plant and does not contain any halophilic bacteria. In another study by Gerrard *et al.* [23] for kinetic modeling of phenol removal by packed bed, it has been reported that mixed culture follow neither zero order nor first order kinetics for phenol biodegradation. This result agrees with our observation. They have shown that using a cocoa-fiber packing in the bioreactor, microorganisms follow Monod type reaction kinetics quite well with K value 100 gm^{-3} with phenol concentration upto 350 mgL^{-1}. But the goodness of fit increased when the kinetics was fitted to substrate inhibition model. If the present study is compared with that result, it can be stated that present study does not require the necessity of fitting the kinetics in substrate inhibition model. Because it is already established in this work that phenol had marginal inhibitory effect on COD removal from organic waste.

Table 2. Value of half saturation constant (K in mg/L) and corresponding value of slope and value of coefficient of determination R^2 for expression of the reaction rate equation in the form of Monod model.

K (mgL^{-1})	Slope[a]	R^2
100	1.2851	0.8547
50	1.1431	0.9594
45	1.1181	0.9606
40	1.0904	0.9612
35	1.0599	0.9612
30	1.0261	0.9603
25	0.9887	0.9584

slope[a] → Slope of Equation (6) when plotted as Qcout + K ln Cout vs. Q Cin + K ln Cin without phenol.

$$y = 1.0599x - 551.75$$
$$R^2 = 0.9612$$
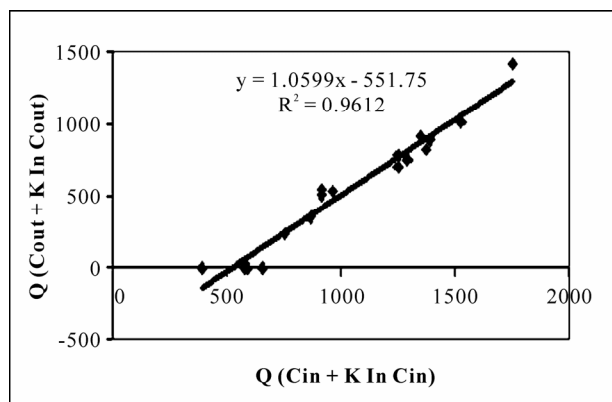
Figure 7. Monod type model for the bioreactor performance with K = 35 mg/L.

This is obvious because the concentration of phenol used in the influent synthetic wastewater was only 50 mgL^{-1}. So, fitting the kinetics in Monod type reaction rate model is quite justified. The K value obtained in our study slightly differs from that obtained by Gerrard *et al.* [23] due to different reasons. Probably their mixed culture consortium contained different microorganisms than that being studied in present investigation. It should also be noted that Gerrard *et al.* have studied the biodegradation of phenol only, where as, present study deals with COD removal in presence of glucose and phenol as the duel carbon sources in the wastewater where both of these organics contribute to COD value.

4. Conclusions

A packed bed bioreactor has been studied for its COD removal efficiency from wastewater. The reactor is packed with glass beads that are covered by biofilm generated by the growth of indigenous mixed microbial culture. The biodegradation efficiency is measured in terms of percentage COD removal from wastewater. The optimum hydraulic and organic loading rates of the bioreactor are found to be in the range of 4-6.4 m^{3}day^{-1}m^{-2} and upto 4.32 kgday^{-1}m^{-3}, respectively. Under this range of operating conditions, the COD removal varied between 100-54%. The performance of the bioreactor decreased marginally under 50 mgL^{-1} phenol charging along with other carbohydrate in the influent wastewater. A mathematical model accounting for reaction kinetics, hydraulic and organic loading rates, is used to describe the steady-state biodegradation of organic matter in presence and absence of phenol, for the bioreactor. In spite of many simplification assumptions made in the mathematical model, our experimental data fits to that model quite satisfactorily. It has been also observed that the rate of biodegradation by the mixed microbial culture in the biofilm followed Monod type rate equation combining a first order and zero order rate expression. By fitting data

in the model, half saturation constant value for biodegradation is calculated to be in the range of 25-35 mgL^{-1}.

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List of Symbols

C	Kgm^{-3}	COD concentration
C_{in}	Kgm^{-3}	COD concentration at reactor inlet
C_{out}	Kgm^{-3}	COD concentration at reactor outlet
Q	m^3s^{-1}	Volumetric flow rate of wastewater
V	m^3	Packed Bed Volume
e	-	Void fraction in packed bed
a	Kgm^{-3}	Dry weight of biomass/volume of packing
r	s^{-1}	COD removed time $^{-1}$biomass concentration^{-1}
k	s^{-1}(zero order) $Kgm^{-3}s^{-1}$ (first order)	Rate constant of reaction
K	Kgm^{-3}	Half velocity constant

Scientific
Research

Perfection of the Electrochemical Method of Sewage Treatment

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Abstract

Problems of the given research are to study the dependence of electrochemical oxidation degree, sewage disinfecting and a filtration of the fish-processing enterprises against time in the presence of sea water. Given tasks are solved by means of sewage processing in static electroflotate with titanic modified ORTA (the orta-ruteniego-titanic anode) by sea water adding of a certain concentration as coagulator.

Keywords: Sea Water, Coagulation, Modified Orta, Electrochemical Oxidation

1. Introduction

Now days an actual environmental problem is clearing and recycling of sewage, formed at various stages of technological processes of the enterprises. Pollutants continue to arrive in water objects, despite of all measures and the methods applied to sewage treatment. The most dangerous pollutants are heavy metals (HM) and organic substances (fibers, fats, dyes etc.).

Last years the tendency of physical and chemical modernization of methods of sewage treatment of the food enterprises from albumens with the aim of their recycling as valuable additives of animals, birds, and fishes forage [1] was outlined. The choice of the sewage clearing scheme of any enterprise depends on many factors: sewage quantities, economic expediency of admixture extraction from sewage, requirements to quality of the cleared water with the purpose of using it in the systems of water supply circulating.

The technology of the household sewage clearing, is based on sea water using, according to it the device for liquid waste dumping into the sea is applied; the containing of the pipeline for liquid waste giving to land station, located on seacoast, and the by-pass pipe which is located on a sea-bottom to rock the sea water into the pool in which there the mixing of a waste with sea water in the set parity [2] is known. Absence of disinfecting system and low degree of drains clearing is the deficiency of this method.

The way of sewage treatment including coagulation of albumenous products with anode oxidation usage and its

subsequent branch coagulate [3] is also known. The process is carried out in two stages to increase the degree of clearance. The way concerns sewage of potato-starch manufactures. However this way does not satisfy sewage treatment requirements of fish processor enterprises. Since, the chemical compound of sewage from processing of hydrobionts is more combined; it is impossible to achieve sufficient clearance from inorganic admixture and lipid contained components in the known way.

There is the technology of sewage treatment of textile factory with use of electrochemical processing with an iron or aluminum electrode and its further additional clearance by polymer [4]. But this technology was not applied for clearing of the food enterprises waters. Also declared degree of clearance is not sufficient and makes 70-80%.

The closest way by technical essence is the way of sewage treatment of fish manufactures by means of sewage processing in electroflotate with ferrous-coal electrodes, with usage of sodium chloride as coagulate and further subsequent additional cleaning by means of the activated form of tseolite [5]. However the known way demands a considerable quantity of the coagulator and the clearing percent after electro oxidation is insufficiently high and makes no more than 70%.

2. Material and Methods

Problems of the given research are to study the dependence of electrochemical oxidation degree, sewage disinfecting and a filtration of the fish-processing enterprises

against time in the presence of sea water. Given tasks are solved by means of sewage processing in static electroflotate with titanic modified ORTA (the orta-ruteniegotitanic anode) by sea water adding of a certain concentration as coagulator.

The influence of time and sea water concentration on coagulation and electro oxidation process on sewage was investigated by us. Sizes of TOC with different concentration of sea water with ORTA were compared to define optimum conditions of clearing at different stages of process.

Sea water is effective sediment of a great number colloidal and dissolved organic pollutants on the account of the high maintenance of magnesium salts and calcium which easily remove a charge of colloidal particles and give low dissolved connections with a great number of polluting substances (anion organic and inorganic acids). Besides, the high maintenance of chlorides in sea water allows receiving at certain potential with sufficient concentration a gipohlorit-ion which is an effective oxidizer of organic and inorganic pollutants.

3. Results-Discussion

Results of studying of coagulation conditions of real sewage fish processing enterprises by sea water are presented graphically (rice 1, 2).

From the presented dependences it is visible that optimum concentration of sea water is concentration of 35% and coagulation time is 90 minutes.

In the result it was established that the maximum degree of clearing is reached under condition of the maintenance in electroflotate of 35% of sea water solution and time of carrying out of electro flotation of 90 minutes. At higher parametres clearing degree does not increase. Therefore the further researches were carried out under these chosen conditions.

Results of clearing of real sewage of fish processing enterprises with 35% concentration of sea water and an electrode for electro flotation ORTA are presented graphically on **Figure 3**.

The clearing percent (%ХПК) has made 92%.

4. Conclusions

Having analyzed the obtained data, it is possible to make the conclusion about expediency of clearing and disinfecting of albumin contained sewage of the food enterprises by means of their mixing with sea water (concentration of 35%) in the electro flotation with application of ORTA-IRON electrodes within 90 minutes. As a result, TOC decreases on 92-96%.

After processing of sewage by electro flotation, sediments and denaturized fibers, salts carbon and inorganic
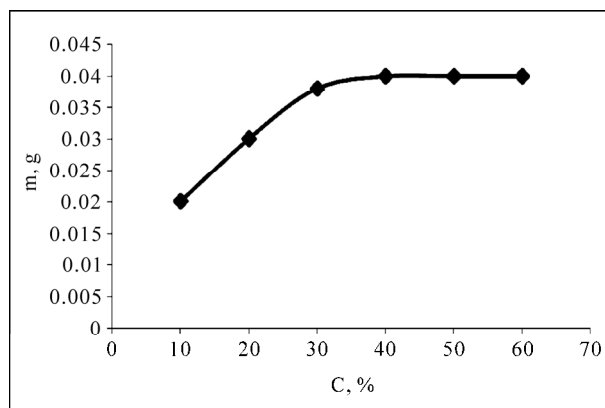
Figure 1. Dependence of weight of sediment on concentration of sea water.
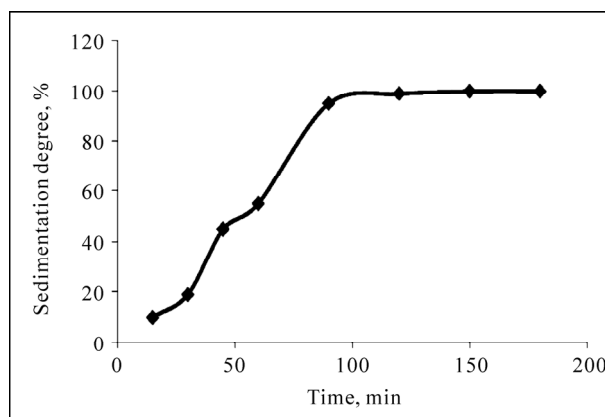
Figure 2. Dependence of degree of sedimentation on time of carrying out of coagulation.
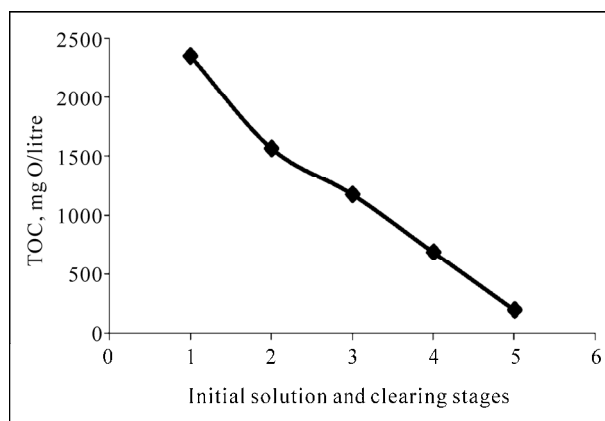
Figure 3. Meaning of TOC of an initial solution and change of TOC at different stages of clearing of real sewage with 35% sea water concentration and an electrode for electro flotation ORTA.

acids are formed by electro oxidation, according to the element analysis data, and spectroscopy

In IR sediment specter (**Figure 4**), allocated after electrochemical processing of waste and modeling water is

Figure 4. IR-spectrum of the deposit allocated after electrochemical processing of sewage.

observed a few distinctive strips of absorption, just strips: 2924, 2853 sm^{-1}–appropriated to valency fluctuations of the co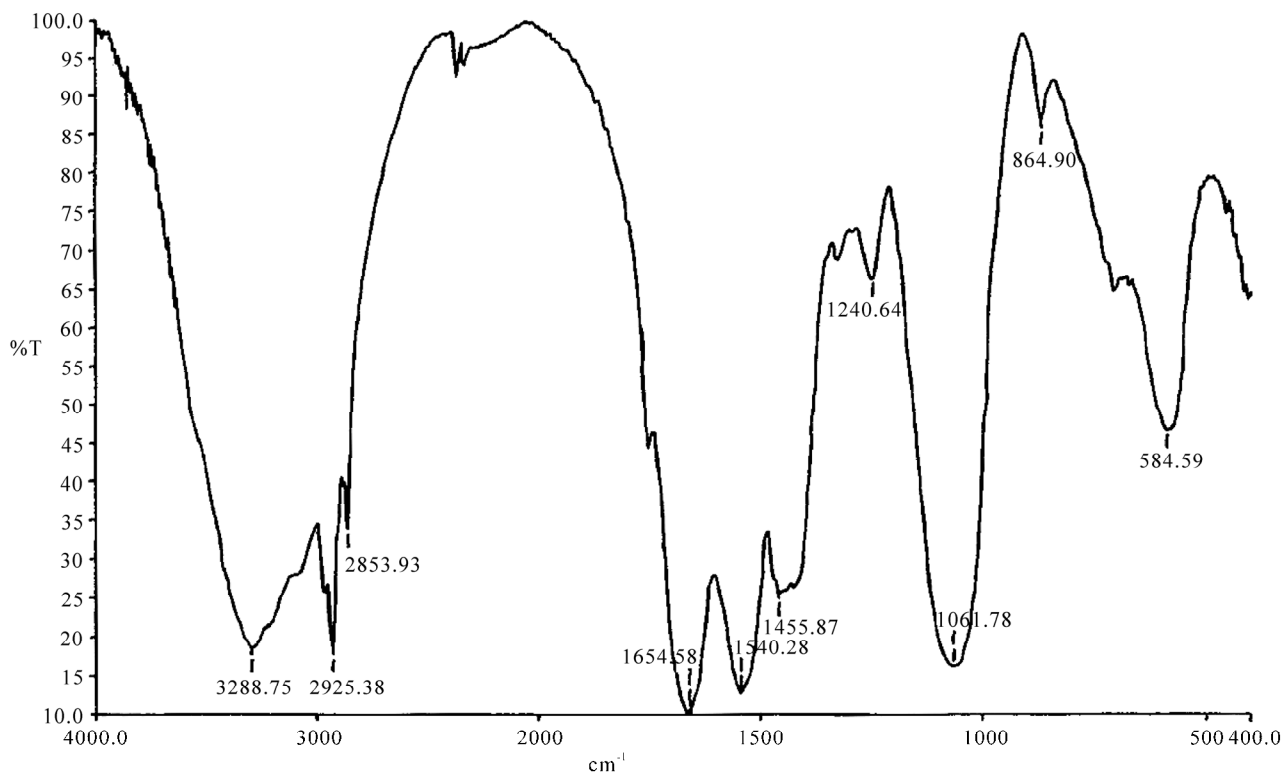nnection C–H, 3400-3200 sm^{-1}–appropriated to valency fluctuations O–H, N–H connections, 1710 sm^{-1}– appropriated to valency fluctuations of connection C=O in carbuncled group, 1630-1650 sm^{-1}–to valency fluctuations in C=O in carbonated connections, 1549 sm^{-1}–to deformational fluctuations N–H, O–H, 1465, 1403 sm^{-1}– to deformational fluctuations C–H, 1150 sm^{-1}, 1096 sm^{-1}– to valency fluctuations C–O in ethereal fragments.

Chlorine is absent after washing of sediments by the distilled water, while before washing by water the chlorine maintenance fluctuated in limits from 1 to 2%.

After electrochemical processing real and modeling sewage were extricated by hexan which does not contain chloride-organic connections. The Chromatographic analysis of extracts has shown the absence of any chloride-organic connections. Thus, it is possible to assert that at electrochemical influence on formation drains of chloride-organic connections does not occur.

Use of the received results is possible in already existing clearing of household sewage technologies of small

enterprises, and as clearings of the fish-processing enterprises drains.

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Comparison of Discharge Duration Curves from Two Adjacent Forested Catchments—Effect of Forest Age and Dominant Tree Species

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Abstract

The effects of forest age and dominant tree species on the water discharge volume have been analyzed by a paired-watershed experiment in two adjacent catchments in Tatsunokuchi-yama Experimental Forest, western Japan. The control period is 1937-1943. The treated periods are 1948-1953, 1968-1977, and 1996-2003. In these treated periods, the forest age or the dominant tree species were different between two adjacent periods. Differences in the discharge duration curves from the two catchments are compared for the control and the treated periods. A significant change in the discharge duration curves is seen in the third treated period (1996-2003) on days with low water, when the forest age difference between the adjacent catchments was 35 years. This is believed to be the result of differences in forest age and forest treatment just after the occurrence of pine wilt disease.

Keywords: Paired-Watershed Experiment, Forest Age Difference

1. Introduction

In recent years, the expectation for the various functions of forests has become wide spread. These functions include those relating to the forest's water budget, such as water resource management and flood prevention. What forest type performs best in terms of these functions is of major interest in forest hydrology. First, two typical indicators can be given for classifying forest types: tree species and stand age.

Bosch and Hewlett [1] compared water budgets in broad-leaved and coniferous forest catchments, and found that deciduous forests perform better in terms of water resource management. This was rebutted by Tanaka and Suzuki [2]. Moreover, some studies have used mathematical modeling techniques to examine forest type and water budget, e.g., the reviews conducted by Komatsu [3] and Komatsu *et al.* [4].

On the other hand, Kuczera [5] provided a curve to show the relationship between forest age (extending up to 200 years) and annual water discharge volume for forest catchments in Australia dominated by eucalyptus and ash. Also, Kosugi and Katsuyama [6] reported change over time in evapotranspiration in Japanese cypress stands. However, there have been few studies of the rela-
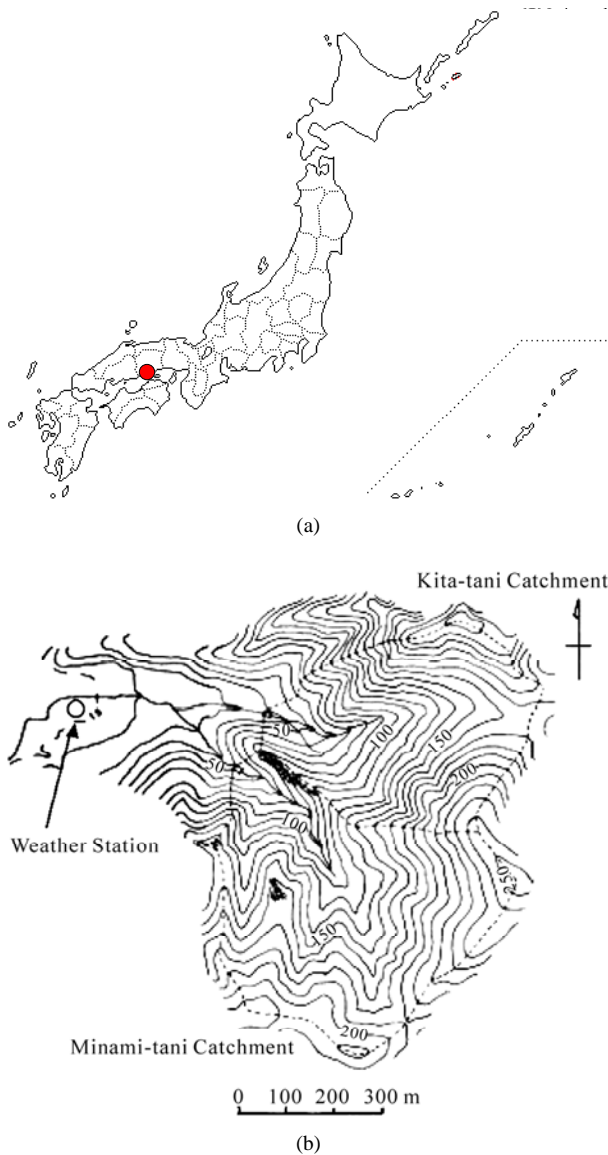
tionship between stand age and water discharge.

Tatsunokuchi-yama Experimental Forest, located in western Japan, has two very similar adjacent forested catchments. The forest in one catchment has been dominated by deciduous broad-leaved species since 1947, and it has existed without disturbances. The forest in the other catchment has a history of forest fires and the occurrence of pine wilt disease. This catchment's species has varied with time between broad-leaved and coniferous trees. Thus, these two catchments areas have forest features that are significantly different. The effects of forest age and dominant tree species on the water discharge volume has been analyzed and reported in this paper by comparing the discharge volumes during each of the three periods as the differences in forest features in the two catchments have changed with time.

2. Method

2.1. Experimental Forest

The Tatsunokuchi-yama Experimental Forest is located at 34°42' N, 133°58' E (**Figure 1**). Annual precipitation and mean air temperature are approximately 1200 mm

tion was measured at an adjacent weather station using a rain gauge.

2.2. Forest History

An overview of forest history in the test area is shown in **Table 1**. The Tatsunokuchi-yama Experimental Forest was established in 1937 to conduct paired-watershed experiments. Red pine (*Pinus densiflora*) was the dominant species at the site in 1937. However, the forest began declining in 1940 as a result of pine wilt disease, and timber was harvested in all areas of the KT and MN watersheds in 1944-1947 and 1944-1945, respectively. Natural regrowth of red pine, oak (*Quercus serrata*), and bamboo (*Pleioblastus chino*) occurred in both watersheds. Deciduous broad-leaved trees have since dominated KT, but the vegetation in MN has been repeatedly disturbed over the years.

In 1954-1957, brush cutting was done over 19.5 ha in the MN catchment. In 1959, 22.3 ha of forest were lost because of forest fire. The burnt area was planted with Japanese black pine in 1960, but in 1978-1980, the black pines were completely destroyed by pine wilt disease. After that, the area was left to recover naturally, and the natural growth consisted primarily of broad-leaved trees.

Changes in stand volume since 1958 are shown in **Table 2**.

Table 1. Vegetation history in KT and MN catchments and analysis period definition.

Year	KT	MN	Analysis Period
1937	Pine Forest	Pine Forest	Control Period
1942			
1947	Clear Cut	Clear Cut	
1952		Deciduous Forest	Treated Period I (Δyear = −2)
1957		Deciduous Forest (Brush Cutting)	
1962		Deciduous Forest	
1967		Forest Fire	
1972		Pine Forest	Treated Period II (Δyear = 13)
1977			
1982		Pine Wilt Desease	
1987			
1992		Deciduous Forest	Treated Period III (Δyear = 35)
1997			
2002			

Figure 1. Location (a) and topography (b) of Tatsunoku-chi-yama Experimental Forest.

and 14.3°C, respectively. The seasonal distribution of precipitation shows little precipitation and almost no snow in the winter, while precipitation is heavy in the months of June, July, and September because of the effects of the rainy season and hurricanes. Precipitation is low compared to other locations in Japan, and the soil can enter a markedly dry state in the summer. Geologically, the area is part of the Chichibu Paleozoic strata, and the soil is a clay soil with significant admixture of gravel classified as a clay loam. The adjacent two catchments are Kitadani (KT, 17.3 ha) and Minamidani (MN, 22.6 ha), and are mainly underlain by Paleozoic formations. Discharge and precipitation have been measured at or near the site since 1937; discharge was measured in the forest using a wire gauge, and precipita-

Table 2. Historical record of stand volume in Tatsunoku-chi-yama Experimental Forest.

(Unit: m³ ha⁻¹)

Year	Minami-tani	Kitatani	Reference
1958	0.9	-	[19]
1963	1.3	11.7	[19]
1968	2.1	73.1	[19]
1973	22.1	102.8	[19]
1978	43.4	135.4	[19]
1998	83.9	158.4	[14]
2005	135.4	201.6	[18]

Many studies have been conducted in the Tatsunoku-chi-yama Experimental Forest. Fujieda *et al*. [7] and Abe and Tani [8] compared the annual discharge volumes from KT and MN. They found MN to have the larger discharge volumes in 1960-1967 and 1981-1996, just after the disturbances by forest fire and pine wilt disease, respectively. In contrast, KT had larger annual discharge volumes than MN in other periods when forest canopy is closed in both catchments. An increase in discharge after periods of forest fires and pine wilt disease has been reported in Fujieda *et al*. [7], Abe and Tani [8] and Tamai *et al*. [9].

2.3. Paired-Watershed Experiment

In KT, forest growth has been consistent since 1948. In MN, on the other hand, the forest was partially lost because of forest fire in 1960 and pine wilt disease in 1978-1980. Therefore, the value obtained by subtracting the MN forest age from the KT forest age (Δyear) varied from −2 years to 13 years to 35 years (**Table 1**). One purpose of this study is to evaluate the effects of forest age on the discharge duration curve using a paired-watershed experiment to compare changes due to Δyear in the relative relationship of the discharge duration curves for KT and MN. KT is taken to be the control catchment (undisturbed), and MN the treated catchment.

Factors affecting the discharge duration curve are classified into three main types: 1) precipitation pattern, 2) vegetation, and 3) territorial functions such as topography and geology. In a paired-watershed experiment, it is possible to cancel out differences in precipitation pattern by comparing the discharge duration curves from two adjacent catchments [1]. If the forests of both catchments are in the same state during the control period, then the effects of vegetation are canceled out. Therefore, the

relative relationship of the discharge duration curves in the control period is regarded as being attributable to differences in territorial functions.

Differences arose in the forests of the two catchments during the treated periods. Therefore, the relative relationship of the discharge duration curves from the two catchments differed from their relative relationship in the control period. By evaluating changes in the relative relationship in the control period and treated periods, it is possible to clarify the effects of the forest differences arising during the treated periods on the discharge duration curves.

In this research, the control period is from 1937, when the experimental forest was established, to 1943, the year before timber harvesting began in both catchments. There were some differences in forest vegetation between the two areas in 1937. In KT, one part (~5 ha) was grass in a previously harvested area, and the rest was natural stands of old/middle-growth red pine. In MN, there were new-growth stands (~6 ha) and old/middle-growth stands (~10 ha) of natural red pine, and a very small area (~1 ha) of planted Japanese cypress stands. However, the stand age of the main stands of old/middle-growth red pine was sufficiently large, so that the difference in stand age can be regarded as non-existent compared to the treated periods described below.

Timber harvesting ended in 1947 in KT and 1945 in MN. Consequently, Δyear from 1948 until 1958, the year before the wildfire, is −2. Incidentally, Tamai [10] has reported that brush cutting was conducted over 19.5 ha in 1954-1957, and effects of this on the discharge duration curve are significant in that period. Therefore, the period 1948-1953, excluding the period after 1954, is taken to be treated period I with a Δyear of −2.

In 1960, Japanese black pine was planted in the part of MN burnt by forest fire. Therefore, Δyear is 13 for the period from 1960 to 1977, the year before the pine wilt disease occurred. Fujieda *et al*. [7] and Tamai *et al*. [9] have evaluated the effects of forest loss in the period 1960–1964, taking that as the period affected by forest fire. Hence, the subsequent period, 1965-1977, was taken to be treated period II with a Δyear of 13. However, since daily discharge was not observed for some days in 1967 and 1974, those years were excluded from the analysis.

Vegetation in the area of MN where the black pines were completely destroyed in 1980 was left to recover naturally as a deciduous broad-leaved forest. Therefore, the Δyear after 1981 is 35. Abe and Tani [8] and Tamai *et al*. [9] have evaluated the effects of forest loss in the period 1981-1984, taking that as the period affected by pine wilt disease. On the other hand, there was a disturbance in 2004 where about 1.7 ha of forest in MN was lost due to wind-toppling. Thus, the period 1996-2003 is taken to be treated period III with a Δyear of 13. However, since daily discharge was not observed for some

days in 1997, those years were excluded from the analysis.

Values for daily discharge in KT and MN were obtained from Forest Experiment Station Ministry of Agriculture and Forestry [11], Forest Influence Unit and Okayama Experimental Site [12], Forest Influence Unit and Okayama Experimental Site [13], Goto *et al.* [14] and Tamai *et al.* [15].

2.4. Discharge Duration Curves

In a discharge duration curve, the 365 daily discharges for one year are arranged in descending order. The left side of the curve indicates daily discharges at times of high water, and the right side indicates daily discharges at times of low water.

The discharge duration curves for KT and MN are compared. A linear regression with a high correlation (Equation (1)) can be obtained between the two catchments in the control period.

$$Q_m \text{ (year, i)} = a_i \, Q_k \text{ (year, i)} + b_i \qquad (1)$$

Here Q_* (year, i) indicates the discharge volume (mm day^{-1}) for the ith day of the discharge duration curve for the given year, and a_i and b_i indicate the slope and intercept, respectively, of the linear regression.

Q_{mcal} (year, i) is calculated using Equation (2) by substituting the observed discharge volume from KT in the treated period (Q_{kobs}(year, i)) into Equation (1) obtained for the control period, and can be regarded as an estimated value for the discharge volume from MN expected when the forests in both catchments were in the same state (*i.e.*, had the same forest age and tree species in this study).

$$Q_{mcal} \text{ (year, i)} = a_i \, Q_{kobs} \text{ (year, i)} + b_i \qquad (2)$$

ΔQ_m (year, i) is calculated with Equation (3) and indicates the change in discharge volume because of the difference in the forest states of MN and KT, in terms of age or tree species.

$$\Delta Q_m \text{ (year, i)} = Q_{mobs} \text{ (year, i)} - Q_{mcal} \text{ (year, i)} \qquad (3)$$

Here Q_{mobs} (year, i) indicates the observed discharge volume from MN during the treated period.

If the water year is taken from April until March of the following year in the Tatsunokuchi-yama Experimental Forest, then there is only one dry season in one water year, and correlation between the discharge volumes from both catchments increases [16]. Therefore, this study takes one water year from April to May of the following year. For example, the discharge duration curve indicated by year = 1997 is for the period from April 1997 to March 1998.

3. Results

3.1. Relative Relationship of Discharge Duration Curves from KT and MN during the Control Period

Figure 2 shows a_i, b_i, and the correlation coefficient of the regression line found for Equation (1) for the control period. The value $b_1 = -2.5518$ was extremely small compared to other values and could not be shown in **Figure 2(a)**. The correlation coefficients for i = 3 and 26 were extremely small (0.9418 and 0.9479, respectively), but correlation coefficients for all other days were 0.95 or higher, and thus the correlation is sufficient for estimating Q_{mcal} (year, i) using Equation (2).

Figure 3 shows curves that average the respective discharge duration curves from MN and KT over the 7 years of the control period. The difference between the two is not clear, but the discharge duration curve for MN is located just slightly above that for KT. The difference is clearly observable in the range i = 100 – 200. This difference is caused by the difference in territorial functions such as topography and geology.
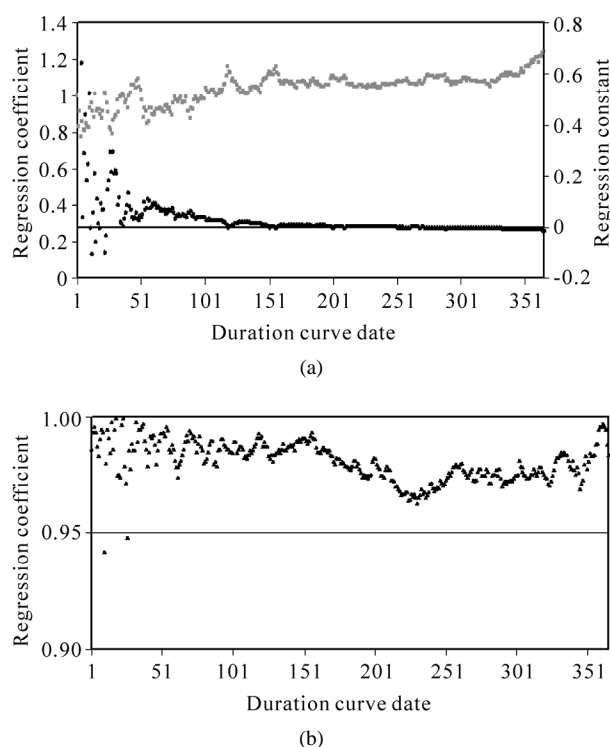

(a)

(b)

**Figure 2. Regression lines between daily discharge volume in discharge duration cuves from KT and MN catchments during control period. (a) Regression coefficient (dark point with left-axis) and regression constant (black point with right axis). The regression constants at i = 1, 2 and 3 are out of range in this figure to be –2.5518, -0.6036 and –0.6329, respectively; (b) Correlation coefficient.**
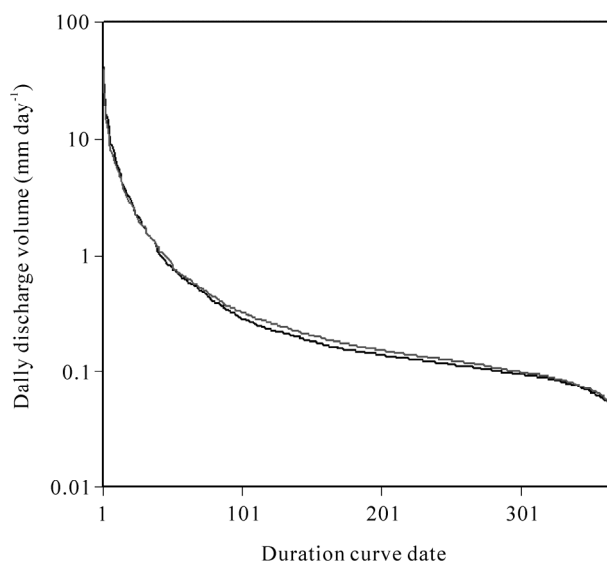
Figure 3. Averaged discharge duration curves during control period. Black line; KT. Gray line; MN.

3.2. Differences in Discharge Duration Curves in Each Treated Period

Figure 4 shows the relationship between Equation (2), and Q_{kobs} (year, i) and Q_{mobs} (year, i) in the control period and treated period for i = 3, 35, 95, 185, 275, and 355. The X-axis indicates Q_{kobs} (year, i) and the Y-axis indicates Q_{mobs} (year, i). Of the three lines, the thick line in the center indicates Equation (2), and the thin lines on both sides indicate the range of the significance level at 90%. If white points are plotted to the upper left side on the regression line, it indicates that the daily discharge volume from MN increases because of the difference between the forested states in both catchments; if those points are plotted to the lower right, it indicates that the daily discharge volume from MN decreases. When white points are plotted far away from the regression line, it indicates that the change in the daily discharge volume from MN is large and the effect is significant. This is caused by the difference in the forest age and dominant species. In other words, white points plotted to the outside of the thin lines indicate that the change compared to the control period is significant at a significance level of 90%.

As a general trend, there are a few points plotted to the lower right of the graph, when i is in the 3–95 range. When i is in the 185–355 range, however, the number of points plotted to the upper left of the graph increases. This means that the relative relationship of Q_{kobs} (year, i) and Q_{mobs} (year, i) in the control period and treated periods varies depending on the size of the daily discharge volume.

It is possible to determine a hypothetical discharge duration curve from MN in each year of the treated period on the basis of the value of Q_{mcal} (year, i) calculated using Equation (2). This is the potential discharge duration curve that would likely have been obtained if the MN forest had the state as KT, without losses due to forest fire in 1959 and pine wilt disease in 1978–1980. **Figure 5** shows the average of the potential discharge duration curves for each of the treated periods I, II, and III, together with the actual observed discharge duration curves. The area between the two discharge duration curves indicates the change in the curves that is thought to have occurred because the stand state is different between MN and KT. Although it is not shown in **Figure 5**, the relationship between the potential discharge duration curve for MN and the observed discharge duration curve for KT is the same as that between the discharge duration curves observed for MN and KT in the control period shown in **Figure 3**. In other words, the discharge duration curve observed for KT is located slightly below the potential MN curve. Therefore, the difference in the curves, which is thought to be due to the different forest state in the MN and KT catchments, becomes somewhat smaller than the difference in the actual observed curves of the two catchments.

The difference between the two discharge duration curves shown in **Figure 5** is not clearly significant for treated period I. For treated period II, the difference is barely significant in the range i > 300. For treated period III, on the other hand, the difference is clearly significant in the range i > 100. However, the vertical axes in **Figure 5** are on a logarithmic scale, and thus it is difficult to see any difference in the range where i is small. Therefore, the average values of ΔQ_m (year, i) for i = 3, 35, 95, 185, 275, and 355 are shown in **Table 3**. A positive value indicates that the discharge volume from MN is calculated to increase because of the difference in forest state between MN and KT. Overall, ΔQ_m (year, i) exhibits a tendency to increase as Δyear and i increase. However, the values found in this study are extremely small compared to ΔQ_m (year, i) in cases where the forest was destroyed by forest fire or pine wilt disease. Exceptions to this are i = 275 and 355 in treated period III.

Figure 6 shows the changes in the ratio (percentage) obtained by dividing the values of the discharge duration curve observed for MN by the values of the hypothetical discharge duration curve (Q_{mobs} (year, i)/Q_{mcal} (year, i)). When this value is above 100%, it means that the discharge has increased because of the difference in forest state between MN and KT, and conversely, when the value is below 100%, it means that the discharge has decreased for the same reason. Q_{mobs} (year, i)/Q_{mcal} (year, i) tended to increase as Δyear increased. In terms of the range of i, where Q_{mobs} (year, i)/Q_{mcal} (year, i) exceeds 100%, there is a gradually increasing number of days exceeding 100%—none for treated period I, i > 169 for treated period II, and i > 50 for treated period III. However, except for treated period III, the value of Q_{mobs} (year, i)
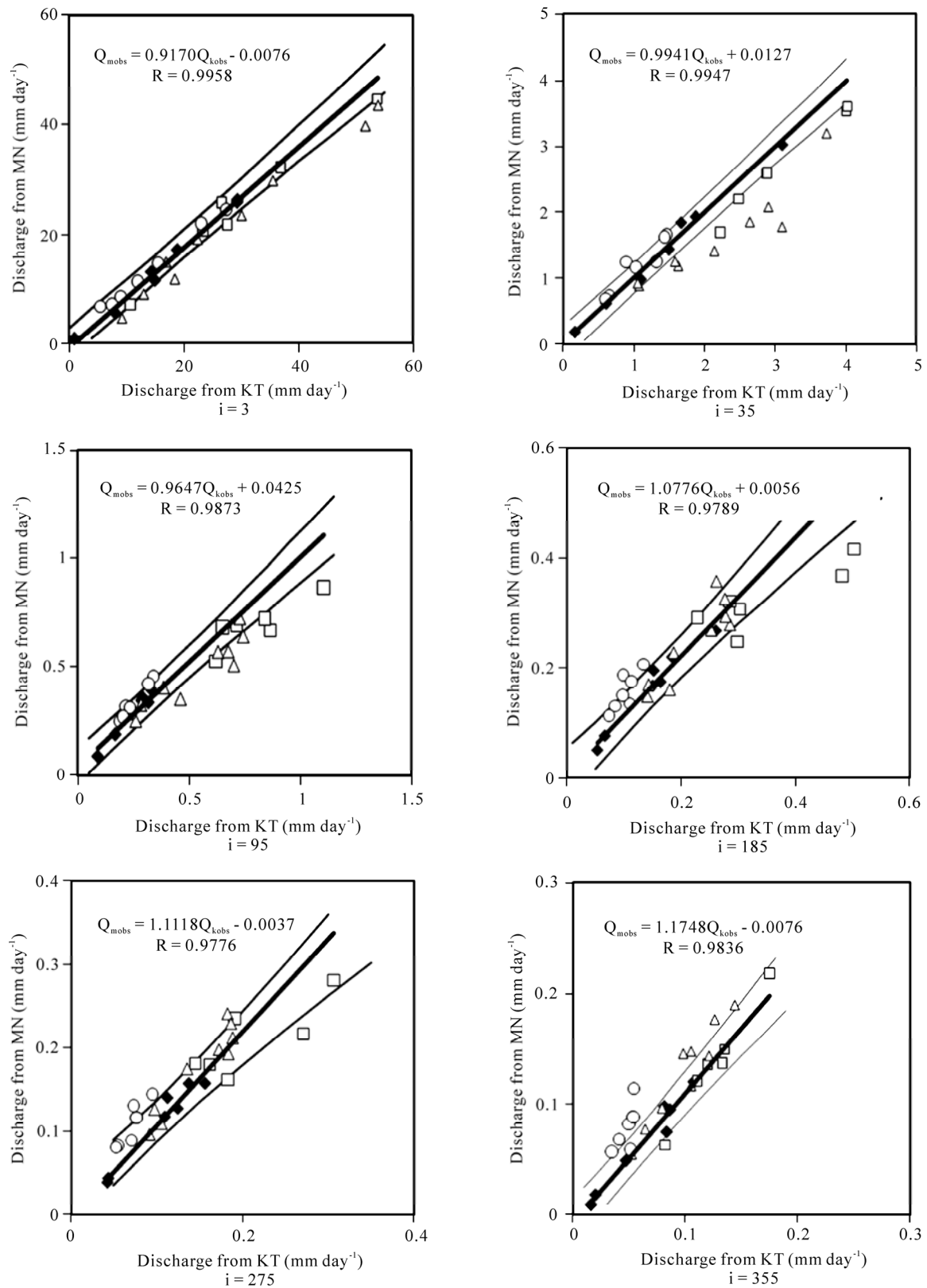
Figure 4. Relationships between observed daily discharge volumes from KT and MN. Black diamond; Control period, White square; Treated period I, White triangle; Treated period II, White circle; Treated period III, Thick line; Regression line in control period, Thin lines; Significant range at 90% reliability.
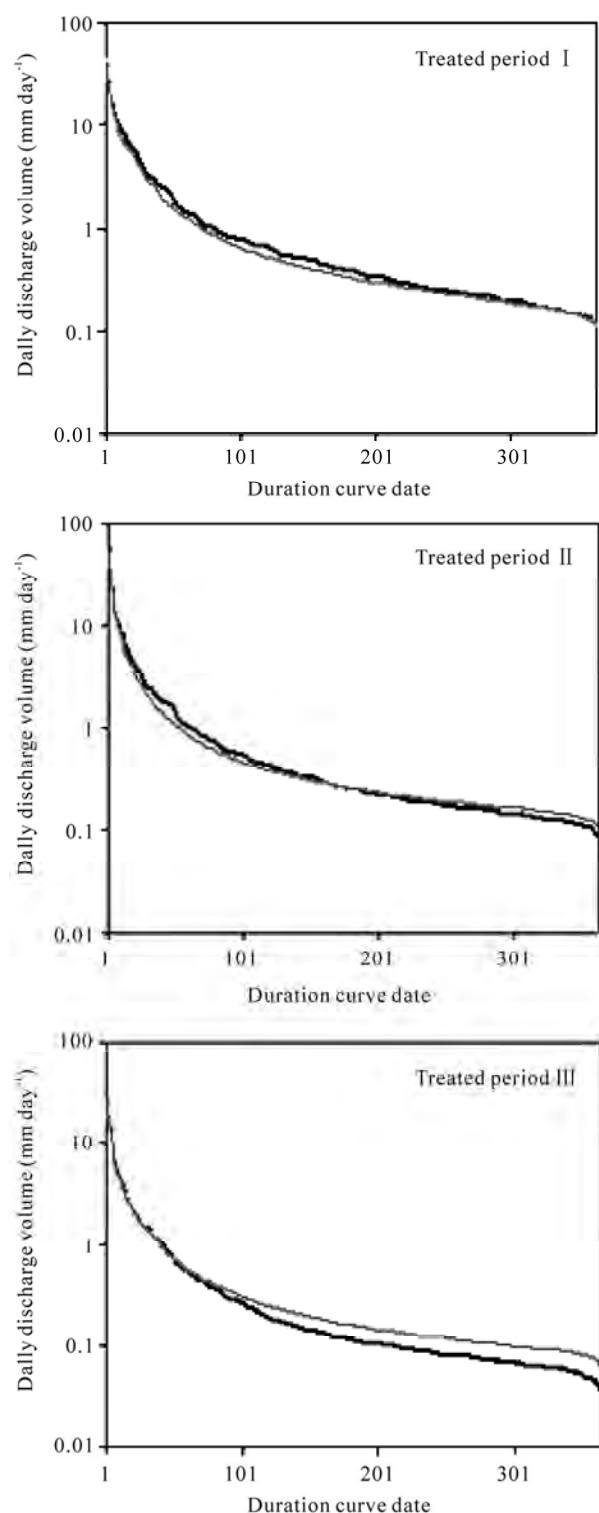
Figure 5. Increased averaged discharge duration curves in each treated periods caused by younger forest age or forest disturbances. Gray line; Observed duration curve from MN. Black line; Calculated duration curve from MN This line is also regarded as the potential curves from MN when same Equation (1) would be adaptable even in the treated periods.
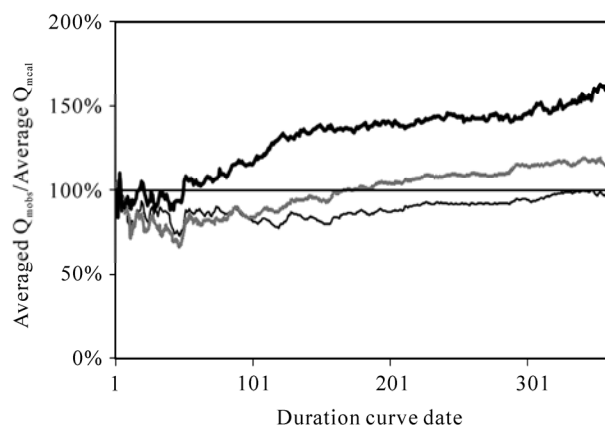
Figure 6. Estimated increased ratio of daily discharge compared with the control period caused by younger forest age or forest disturbances. Thin black line; Treated period I, Gray line; Treated period II, Thick black line; Treated period III.

Table 3. Estimated increased volume from MN compared with the control period caused by younger forest age or forest disturbances.

N	I	II	III	Forest Fore[*]	Pine Wilt Disease[*]
Year	1948-1953	1968-1977	1996-2003	1960-1966	1981-1994
3	−1.259	−3.070	−2.639	29.189	21.898
35	−0.370	−0.586	−0.042	2.556	2.046
95	−0.119	−0.082	0.043	0.227	0.158
185	−0.058	0.002	0.041	0.108	0.098
275	−0.021	0.013	0.033	0.066	0.059
355	−0.003	0.019	0.030	0.048	0.056
Averaged Annual Precipitation	1331.2	1236.6	1134.2	1275.0	1123.6

$/Q_{mcal}$ (year, i) is close to 100%. That is, the change was small. In the range of $i > 307$ for treated period III, the value of Q_{mobs} (year, i)$/Q_{mcal}$ (year, i) became larger than 150%. This means that the discharge volume from MN during the about 60 days of low water was about 1.5 times greater than the reference. The value of Q_{mcal} (year, i), used as the reference, is the discharge volume from MN expected if the forest was the same as KT, and had not been destroyed due to events such as forest fire and pine wilt disease. Therefore, if the value of Q_{mcal} (year, i) is taken to be the standard, then there are about 60 days of low water. However, since discharge volume in the time of low water has a smaller absolute volume, there is

little change in the annual discharge volume. In terms of the average value for treated period III, Q_{mobs} (year, i) was 229.832 mm year^{-1}, but ΔQ_m (year, i) was only 1.402 mm year^{-1}.

4. Discussion

Discharge volume from MN is calculated to be 1.5 times greater during the about 60 days of low water in treated period III. There are three possible reasons: 1) The effect of the forest loss because of pine wilt disease has continued in treated period III because the vegetation was left to recover naturally. 2) The forest age in KT is larger in treated period III than other periods. Thus, the large Δyear causes the large difference between the discharge volumes from KT and MN. 3) In treated periods I and III, the dominant species in MN was deciduous broad-leaved trees, the same as in KT. However, in treated period II, it was coniferous evergreen trees, different from that in KT. The difference in the dominant species might cause the difference in discharge volumes between the catchments. On the basis of this hypothesis, ΔQ_m (year, i) in treated period II would be as large as in treated period III. The three possibilities are discussed below.

4.1. Vegetation Treatment Differences after Disturbances

In the case of treated period II, Japanese black pine was planted in the damaged area in MN in 1960 just after the forest fire. It took five years for the discharge volume from MN to recover from the effects of the forest fire [7]. On the other hand, in the case of treated period III, the vegetation was left to recover naturally after the pine wilt damage. It took 16 years for the annual discharge volume from MN to recover to almost the same discharge volume as KT. However, the low (i = 265) and drought (i = 355) discharges had not recovered even by 2004 [10]. The difference in how the vegetation was treated after the loss of the forest may have caused the difference in the discharge volume between treated period III and the other treated periods.

4.2. Differences in Forest Age

If the large value of Δyear causes the large difference between the discharge volumes from both catchments, this suggests that the water loss effect of the old forest in KT has changed from that when it was younger. Kuczera [5] found the relationship between the forest age and annual discharge volume for forest catchments in Australia that were dominated by eucalyptus and ash, and proposed a curve where water discharge reaches a mini-

mum with a forest age of 25 years and increases after that. A decrease of 50-400 mm year^{-1} was found at a forest age of 25 years. On the other hand, Kosugi and Katsuyama [6] found seasonal changes in evapotranspiration of a Japanese cypress forest using the short-term water discharge method, and there were no clear differences, in terms of seasonal fluctuations or yearly volume, over a period of 33 years.

In this study, it was calculated that for about 60 days at the time of low water, discharge from KT with an age of 49-56 years was reduced to 2/3 or less of the discharge from MN with an age of 16-23 years in treated period III. In the forest age relationship in the Kuczera curve [5], discharge from a 49-56 year old forest catchment was larger than that from a 16-23 year old forest catchment. This difference may be because the forest growth rates in the KT and MN catchments are smaller than those in the forests studied by [5], and the forest age exhibiting the minimum value is larger.

Vertessy et al. [17] reported that the Sapflow area index (SAI) or Leaf area index (LAI) decrease is the cause of the discharge increase from a forest catchment with the age of 25 year or older. Goto et al. [18] reported the LAI in the KT catchment increased from 7.0 in 1998 to 8.0 in 2005. This LAI increase was concurrent with the increase in forest age in KT, from 50 in 1998 to 57 in 2005. Thus, the evaporation rate is supposed to increase with forest age. Also, in the Kuczera curve, there is a reduction of 50-400 mm year^{-1} when the forest age is 25 years. However, the increase in this study was only 1.402 mm year^{-1}, and thus no large change was seen. This agrees with the results of [6].

The forest in KT grew even when the forest age was 50-57. Thus, the maturity might cause the low and drought discharge volume from KT to be larger than that from MN in treated period III.

4.3. Differences in the Dominant Species

The dominant tree species of MN in treated period II was Japanese black pine, which is different from KT. Therefore, in addition to differences in forest age, there is a possibility that effects due to differences in dominant tree species are also included in ΔQ_m (year, i) for treated period II. [4] conducted model calculations of annual evapotranspiration of three catchments, including MN in 1971-1977 and 1996-2000, and found that evapotranspiration of broad-leaved trees was almost equal to that of young coniferous forests. The forest age in MN in treated period II was young (8-18 years). Therefore, it is thought that differences in dominant tree species had little effect on ΔQ_m (year, i) in treated period II. Similarly, the difference in the dominant species in MN between periods II and III had little effect on the large volume of ΔQ_m (year, i) in treated period III.

5. Conclusions

In this study, we found that discharge for about 60 days in the time of low water from KT in treated period III was reduced to 2/3 or less of that from MN of Tatsunokuchi-yama Experimental Forest, Okayama City. Possible causes are 1) vegetation treatment differences after disturbances, 2) differences in forest age, and 3) differences in the dominant species. This study concluded that 3) cannot be the cause. However, 1) and 2) are possible causes. Both are related to forest management practices. This means that periodic and adequate forest treatment is necessary to maintain water discharge at low water events. However, since this study was limited to two catchments and limited samples, it will be necessary to further these studies using an increased number of sampling periods and catchments.

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Scientific
Research

Assessment of Effluents from Associated Match Industries with Emphasis on Bioaccumulation of Heavy Metals in Crab

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Abstract

Heavy metals disposed through anthropogenic activities find their way into aquatic environment through factory effluents. These heavy metals resuspend back into the water column along with the sediments and are known to affect aquatic bioresources. Effluent water samples and crabs were collected along top camp stream to evaluate heavy metal concentrations and were assessed to determine the uptake of heavy metals in crabs. Results show fluctuation in acidity/alkalinity of water samples. BOD, COD, DO, conductivity and organic phosphorous were below permissible limit, and metal concentrations (As, Cu, Cr and Zn) in water samples do not constitute a risk factor for human health. However, concentrations of Cupper (Cu), Iron (Fe), Asenate (As) and Manganese (Mn) in crabs were significantly higher than the level found in the effluent water. Heavy metal poisoning has been identified among people that depend on crabs for their protein. Such effects are viewed by international health organization as attenuation of human health. The toxic elements (As, Cu, Fe and Mn) in the effluent and dissolved solid concentrations including potassium and calcium carbonate may be reduced through resource recovery.

1. Introduction

Effluent discharges from industries constitute a serious environmental threat to water quality and aquatic resources; including biodiversity. Likewise, contaminations of soil by the release of heavy metals as a result of industrial and anthropogenic activities are threat to human health and ecosystem [1,2]. Heavy metals are stable and recalcitrant contaminants of aquatic environments. Some metals ions are required for metabolic activity while, many inadvertently may have concentrations that lie in the narrow range between their essentiality and toxicity. Additionally, others may exhibit extreme toxicity even at low levels under certain conditions [3,4]. As distinct from organic substances, they migrate and accumulate in different components of natural ecosystems (water, soil, bottom deposits and aquatic animals). Most heavy metals are supposed to accumulate in aquatic animals and pass their toxic effects onto the upper links of the trophic chain, including human beings [5]. Conse-

quently, regular monitoring of sensitive aquatic environments is quite necessary.

The study of organisms as pollutant monitors has several advantages over the chemical analysis of abiotic comportments. Chemical, toxicological and ecological approaches have been employed extensively in assessing impacts of heavy metal pollution in aquatic environments. Many such studies have concentrated on birds [6], Fish [5,7] in mussels [8] in other invertebrates [9] and vegetation [10].

The tendency of heavy metals to accumulate in aquatic organisms is of scientific interest not only in heavy metal chemistry but also in environmental studies. In particular, the bioavailability of tissue metal levels in crab; an important staple part of food delicacies in some localities, is an indicator of environmental pollution. Ultimately, the contents and properties of effluents could be responsible for possible environmental impact on different matrices (water column, sediment and biota), and possibly contribute to the characteristics of the receiving water.

The aim of this study was to contribute to environ-

mental effect assessment of effluent discharged by Associated Match industry located in Southwest, Nigeria. In particular we report preliminary results regarding heavy metal concentrations in crab, as a bioindicator of heavy metal biomagnifications.

2. Materials and Methods

The study area was along the Eleyele industrial layout of Ibadan, North-West Local Government, Oyo State. (**Figure 1**). The city lies between latitudes 07°22'30" N and 07°25'50" and longitude 003°2'00" E to 003°55'50" E, at an altitude approximately 1500m above sea level. The climate of the area is influenced by Tropical Maritime (mT) and Tropical Continental (cT) air masses. The mean annual rainfall is 1413 mm, while the mean annual temperature ranges from 22.5℃ to 31.4℃. The soil in the area support tropical rainforest vegetation while Kaolinite is the main clay mineral present [11-13]. The area is located in the lowland rainforest vegetation zone of Nigeria [14]. It is a major industrial section and various untreated or minimally treated wastes are discharged into the stream.

It encompasses the stretch between ten sampling stations. Four of these sampling stations were established along the effluent stream ($P5_o$, $P5_1$, PS_2 and $P5_3$) from the industry. One station was at the confluence of the effluent stream, and adjacent natural stream (Topcamp stream, C_1), three stations were along the TopCamp stream (BC_o, BC_1 and BC_2) before the confluence C_1, and the other two stations after the confluence (AC_1 and AC_2). A portable Global Position System (GPS) set was used to locate the grid coordinates of sampling stations.

Water samples were drawn from each of these locations mentioned above 20 m apart except C_1 (Topcamp stream) because of its topography beneath rock and the surrounding wetland. The samples were analyzed for pH, electrical conductivity, turbidity, total acidity and alkalinity, oxygen demand (BOD and COD) including major ions (Cl^-, SO^{2-}_4, K^+, Na^+, Ca^{2+}). Water samples were filtered through a 0.45mm whatman no 1 filter paper. Water samples (100ml) were acidized to a final concentration of 2% with nitric acid. Two samples were divided into two and portion of each half was spiked with known concentrations (0, 2.5, 5.0 and 10.0 ppm) of the six analytes in order to determine percent recovery. Unspiked samples, blanks and spiked samples were analyzed by Alpha 4 atomic absorption spectrophotometer (AAS).
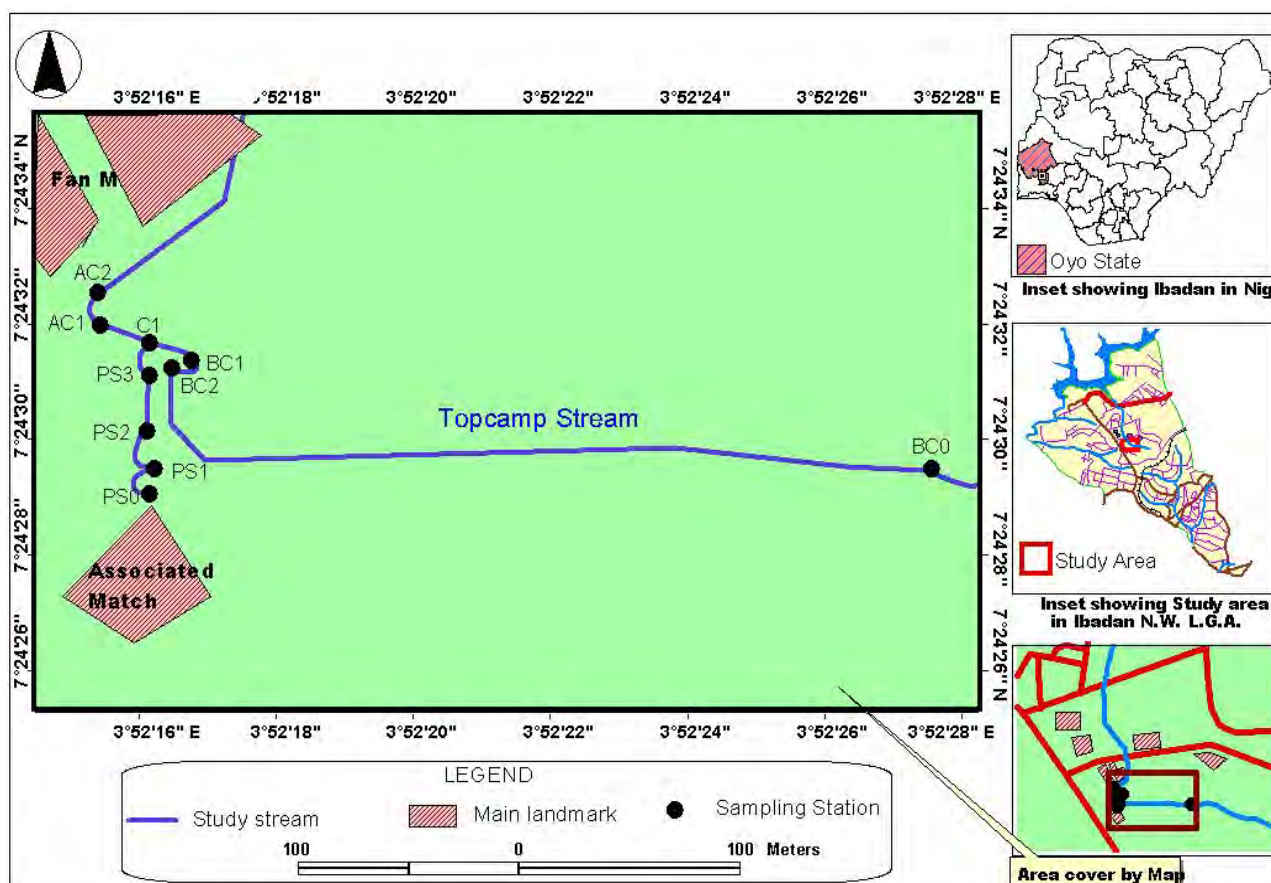

Figure 1. Detailed map of study area showing sampling stations.

The AAS was standardized regularly once after analyzing three samples with matrix-matched standards (Inorganic Ventures, Lakewood, NJ). Standardization was verified with appropriate external standards (Spex Industries Inc., Edison, NJ). Analyte recovery in spiked samples ranged from 92-100%.

At least 20 crabs *Sudanenautes africanus africanus* samples were collected along the Topcamp stream flow. Each crab was excised. The whole internal organs of the crab were removed by steel pliers and the damp weight was measured. All samples were kept at 85℃ for 48h to dry to constant weight. The samples were digested according to the procedure given by US EPA 1996 [15] using concentrated H_2SO_4 and HNO_3 in beakers placed on a hot plate (1:10). The amount of various essential and non-essential elements like As, Cr, Cu, Fe, Mn and Zn in crab samples was determined by multiplying by the dilution factor given in ppm/g. The detection limits were

as follows: 1 mg/L for Fe; 0.01 mg/L for Cu, As, Cr, Mn and Zn.

Statistical analysis: Data were subjected to Barlett's test for homogeneity, followed by analysis of variance (ANOVA). For post hoc comparison Student Newman Keul's test was employed.

3. Results and Discussion

The present study aims in understanding the bioaccumulation patterns and demonstrates the safety of aquatic food as a supplementary source of protein in diet. The results of the physcio-chemical characteristics of the discharged effluents in water course, confluence and quality of ensuring water are summarized in **Table 1**. The pH of the effluent water fluctuated between acidic to slightly alkaline. The temperature of the surface water

Table 1. Physico-chemical characteristics of water quality at various sampling points.

Parameter	$P5_5$	$P5_1$	$P5_2$	$P3_3$	C_1	AC_1	AC_2	BC_0	BC_1	BC_2
pH	6.49	6.43	6.65	6.47	7.76	8.06	8.01	7.65	8.27	8.09
Temperature Air	30	30	30	29	29	29	29	29	28	30
temp.(℃)	0.4	0.8	0.8	1.6	4.4	4.4	2.3	3.6	2.4	2.0
BOD	5.21	5.07	4.14	5.29	2.37	3.83	2.98	1.47	2.06	2.87
COD	7780	2540	3140	3680	1120	840	780	280	680	480
Total Solid	668	620	180	640	420	160	240	160	140	60
TSS	9.32	3.26	4.35	4.70	1.51	1.13	0.94	0.064	0.65	0.67
Conductivity	298.47	198.48	177.27	153.03	43.95	13.65	16.68	13.65	98.49	1.53
Turbidity	2.6	2.0	2.4	3.2	4.8	4.8	3.8	4.4	5.2	3.2
Dissolved	55.17	17.40	21.39	19.88	07.46	22.86	32.31	3.48	08.45	6.46
Chloride	247.3	205	24.24	43.91	30.51	41.63	27.38	0.24	19.96	33.65
Sulphate	0.418	0.458	0.300	0.305	0.516	0.592	0.658	0.120	0.556	0.760
Inorganic PO_4	1.150	490	710	675	205	130	125	1.75	95	40
K^+	34	30	36	40	30	28	24	08	08	1.8
Ca^{2+}	41	25	29	28	16	11	17	2.6	8.25	9.6
Na^+	45.82	51.43	58.03	47.13	48.57	44.77	43.88	1.93	35.90	42.14
Mg^{2+}	73.9	69.8	78.35	65.5	53.55	51.09	28.54	24.43	48.73	37.75
NO_3^-	720	260	540	540	150	70	60	30	50	40
$CaCO_3$										

All the values are in mgL^1 except pH and Electrical conductivity (µS/cm) and phosphate ($µgL^1$); BOD–Biological Oxygen Demand; COD–Chemical oxygen Demand; TSS–Total suspended solid.

was near constant. However, BOD, COD, conductivity, dissolved oxygen and organic phosphorus were below WHO permissible limit [16]. On the contrary, total solid, turbidity, potassium, nitrate, chloride and calcium levels were markedly high at point source. For example, an electrical conductivity of up to 932 μs/cm has no effects, but water with a high salinity can be toxic to most plants and may pose salinity hazard [17]. Additionally high sodium levels contribute to salinity problems and interfer with magnesium and calcium availability resulting in sodium hazard. CaCo₃ representing alkanity of water is more significant than its pH because it takes into account the principal constituency that influence the water's ability to regulate the pH of the water, thus higher CaCo₃ results in lower pH and lower CaCo₃ results in higher pH (**Table 1**).

The common African crab is one of the few species found all year round. Heavy metals absorbed by the crabs have a possibility to get accumulated in different parts of the body and the residual amount can build up to a toxic level as is the case with Cu, Fe, and Mn. The concentration patterns in the water effluent revealed the following order Mn > Fe > Cu > Cr > Zn > As (**Figure 2**) whereas the accumulation of heavy metals in crabs followed different sequence of Ca > Fe > Mn > Cu > As > Cr > Zn (**Figure 3**). The order of accumulation in crab is proportional to various metal deposits in the effluent. The concentrations of heavy metals in unimpacted (upstream) sections of Topcamp are illustrated in **Figure 4**. The crabs, hepatopancreas showed higher concentration of all metals, especially, calcium this condition might be due to the fact that hepatopancreas plays a prime role in binding the metals and act as primary route of excretion.

The water analysis of heavy metals in the study area was compared to the accumulating pattern in hepato pancreas of indigenous crab, *Sudanenautes africanus africanus* (**Figure 2**). Metal concentration of arsenic, chromium, copper and zinc in water samples in this study do not constitute a risk factor for human health and appear to be below the permissible limits set by the UNEP (1993) [18]. However, metal concentration of Cu,
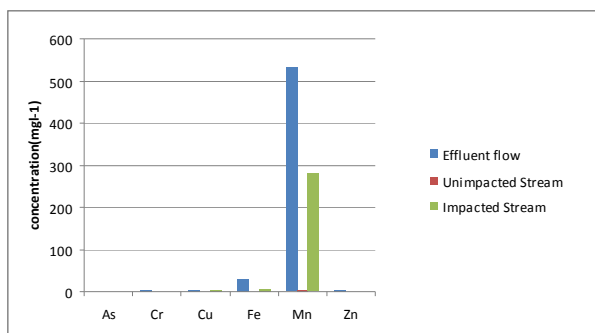

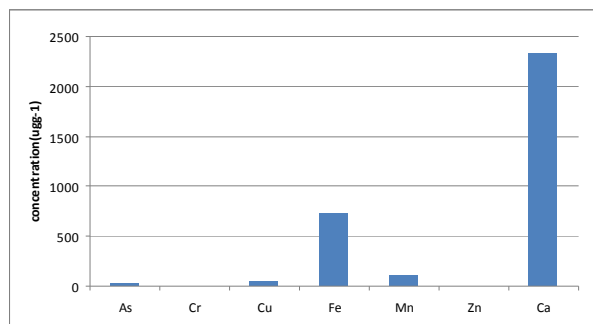**Figure 2. Mean concentration of heavy metal content of water/effluent sample (mgl⁻¹).**

Figure 3. Mean concentration of heavy metals in crab specimen (ugg⁻¹) from impacted section of Topcamp Stream.

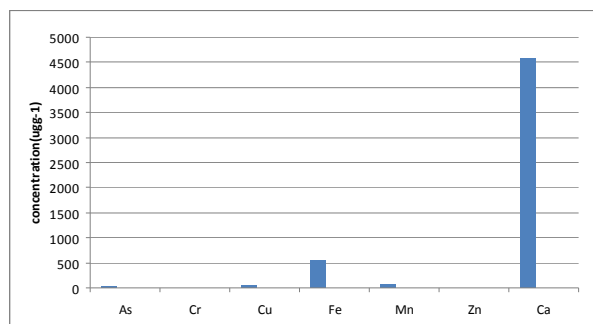**Figure 4. Mean concentration of heavy metals in crab specimens (ugg⁻¹) from unimpacted section of Topcamp Stream.**

Fe and Mn in crabs is significantly higher than the levels found in the effluent water. Consequently, in terms of ecological, crab is irreplaceable bioindicators of the degree of damage to the water environment. Moreover, it is also important to monitor the contamination of crab with heavy metals, because frequent consumption of the contaminated crab presents a very serious health risk. The results of the present study enabled us to determine the background concentrations of these metals in both water and crab. These data will constitute a reference to future studies on the evolution of contamination in this area. On the other hand, a potential danger may occur in the future depending on the agricultural, recreational activities and effluents from the Associated Match Industries.

The crab, *sudanenautes africanus africanus* is economically important and forms a large part of aquatic catch in the area of study. However, heavy metal poisoning had been identified among people that depend on crabs for their protein [19]. The effects of heavy metal contamination are viewed as an international problem because of the effects on human health and ecosystem [20,21]. The concentrations of most toxic elements, other than As, Fe, Cu and Mn are not dramatically excessive. These heavy metals, the high dissolved solids concentration, including potassium and calcium carbonate may as well be withheld for corrective treatment before release

into the aquatic environment.

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Scientific
Research

16-23S rRNA Spacer Region Polymorphism in Gangetic River Water Isolates of *Salmonella*

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Abstract

Salmonella is one of the major pathogenic bacteria present in contaminated water. 16-23S rRNA spacer region has been reported to be polymorphic at serovar level in *Salmonella*. *Salmonella* isolates obtained from Ganges river water were studied for 16-23S rRNA spacer region polymorphism. Thirty three isolates belonging to eight serovars (*S.* Typhimurium, *S.* Abuja, *S.* Pantypridd, *S.* Lagos, *S.* Chinkual, *S.* Zwickau, *S.* Goldenberg and *S.* Oritamerin) were studied for the polymorphism. Out of 33 isolates, 15 different profiles were observed no serovar specific profile. Our findings indicate that 16-23S rRNA spacer region is not specific at serovar level, but can be used for differentiation of different *Salmonella* isolates.

1. Introduction

The Ganges is a major river in Indian sub-continent and is considered to be Sacred and has been declared as National River by Indian Government in Year 2008. The fertile soil of the river basin measuring around one million square kilometers is a key to agricultural economy of the country and supports one of the highest human density populations. The large quantity of pollutant such as untreated sewage material in large volume (approximately one billion liters per day) [1] are disposed off in river Ganges that leads to accumulation of hazardous pathogens like *Shistosoma*, *Escherichia coli* O157:H7, *Shigella and Salmonella* [1-3] and expose the surrounding population to these diseases.

Salmonella is considered a dreaded pathogen causing several diseases such as endocarditis [4], Typhoid and Paratyphoid fever [5], Pneumonia [6] and meningitis [7]. Identification and serovar differentiation are prime requisite for controlling Salmonellosis. Conventional serovar typing is cumbersome and time consuming process [8], but with the development of molecular biology techniques like PCR genome has been targeted for this purpose. In the genome of the bacteria rRNA region has been extensively used for taxonomic purpose [9,10]. In *Salmonella*, 16-23S rRNA region has been studied and reported to be serovar specific [11], as he differentiated many serovars of *Salmonella* using 16-23S rRNA polymorphism. The basis of differentiation of serovars was

based on variation in number and type of t-RNA sequences found within the spacer region of 16-23S rRNA [12]. The present study was undertaken to differentiate eight serovars of *Salmonella* isolated from Ganges river water by targeting 16-23S rRNA region.

2. Material and Method

Thirty three isolates of *Salmonella* were obtained from six different locations (stations) viz, Haridwar, Hastinapur, Kanpur, Garhmukteshwar, Varanasi and Narora. These isolates were serotyped at National *Salmonella* Centre I.V.R.I. (Izatnagar) India, and were characterized as *S.*Typhimurium, *S.* Abuja, *S.* Pantypridd, *S.* Lagos, *S.* Chinkual, *S.* Zwickau, *S.* Goldenberg and *S.* Oritamerin (**Table 1**). Genomic DNA was isolated from all isolates by C-TAB method [13].

3. 16-23 S rRNA Spacer Region PCR

Five μl (40ng) of genomic DNA was used for 50μl of PCR reaction mixture, containing 20 pmol of each primer (Primer1 (5' GAA GTC GTA ACA ACG 3') and Primer2 (5'CAA GGC ATC CAC CGT 3'), 200 μM of dNTPs, 3.0U of Taq polymerase and 1.5 mM final concentration of $MgCl_2$. All chemicals used in PCR were procured from MBI-fermentas. PCR program was as follows-initial denaturation (94℃ for 5 min.) followed

Table 1. Description of *Salmonella* isolates (serovars, place of isolation, band profile and Molecular type) isolated from various locations from river Ganges.

S.No.	No. of isolate	Serovar	Place of isolation	Band Size (base pairs)	Molecular Type
1	G-29	*Salmonella*Typhimurium	Narora	529, 740	M1
2	G-33	*Salmonella* Typhimurium	Narora	529, 740	M1
3	G-36	*Salmonella* Typhimurium	Narora	529, 740	M1
4	G-40	*Salmonella* Typhimurium	Narora	529, 740	M1
5	G-23	*Salmonella* Typhimurium	Narora	529, 740	M1
6	G-28	*Salmonella* Typhimurium	Narora	529, 740	M1
7	G-35	*Salmonella* Typhimurium	Narora	529, 740	M1
8	G-37	*Salmonella* Typhimurium	Narora	529, 740	M1
9	G-38	*Salmonella* Typhimurium	Narora	529, 740	M1
10	G-47	*Salmonella* Typhimurium	Narora	529, 740	M1
11	G-4	*Salmonella* Abuja	Hastinapur	645	M2
12	G-6	*Salmonella* Abuja	Hastinapur	645, 414	M3
13	G-7	*Salmonella* Pantopyroid	Kanpur	645, 414, 334	M4
14	G-12	*Salmonella* Lagos	Haridwar	414, 334	M5
15	G-13	*Salmonella* Lagos	Haridwar	414, 334, 744, 645	M6
16	G-15	*Salmonella* Lagos	Haridwar	645, 414, 334	M4
17	G-17	*Salmonella* chinkaul	Varanasi	645	M2
18	G-19	*Salmonella* Abuja	Hastinapur	645	M2
19	G-22	*Salmonella* Abuja	Hastinapur	645	M2
20	G-25	*Salmonella* Zwickau	Narora	744, 645	M7
21	G-39	*Salmonella* Oritamerin	Narora	645	M2
22	G-1	*Salmonella* Typhimurium	Haridwar	1820, 1487, 750, 603, 532	M8
23	G-10	*Salmonella* Typhimurium	Hastinapur	1820, 1487, 750, 603, 532	M8
24	G-14	*Salmonella* Typhimurium	Garhmukteshwar	1820, 1487, 750, 603, 532	M8
25	G-11	*Salmonella* Typhimurium	Hastinapur	738, 594	M9
26	G-16	*Salmonella* Typhimurium	Haridwar	1820, 1487, 750, 603, 532	M8
27	G-20	*Salmonella* Typhimurium	Garhmukteshwar	1141, 705, 566, 193, 144	M10
28	G-21	*Salmonella* Typhimurium	Garhmukteshwar	2408, 1441, 705, 560	M11
29	G-45	*Salmonella* Typhimurium	Narora	538, 733	M12
30	G-46	*Salmonella* Typhimurium	Narora	538, 733	M12
31	G-50	*Salmonella* Typhimurium	Narora	2435, 1758, 1431, 538, 733	M13
32	G-52	*Salmonella* Typhimurium	Narora	1758, 1431, 538, 733	M14
33	G-55	*Salmonella* Typhimurium	Narora	1291, 538, 733	M15

(a)

(b)

(c)

(d)
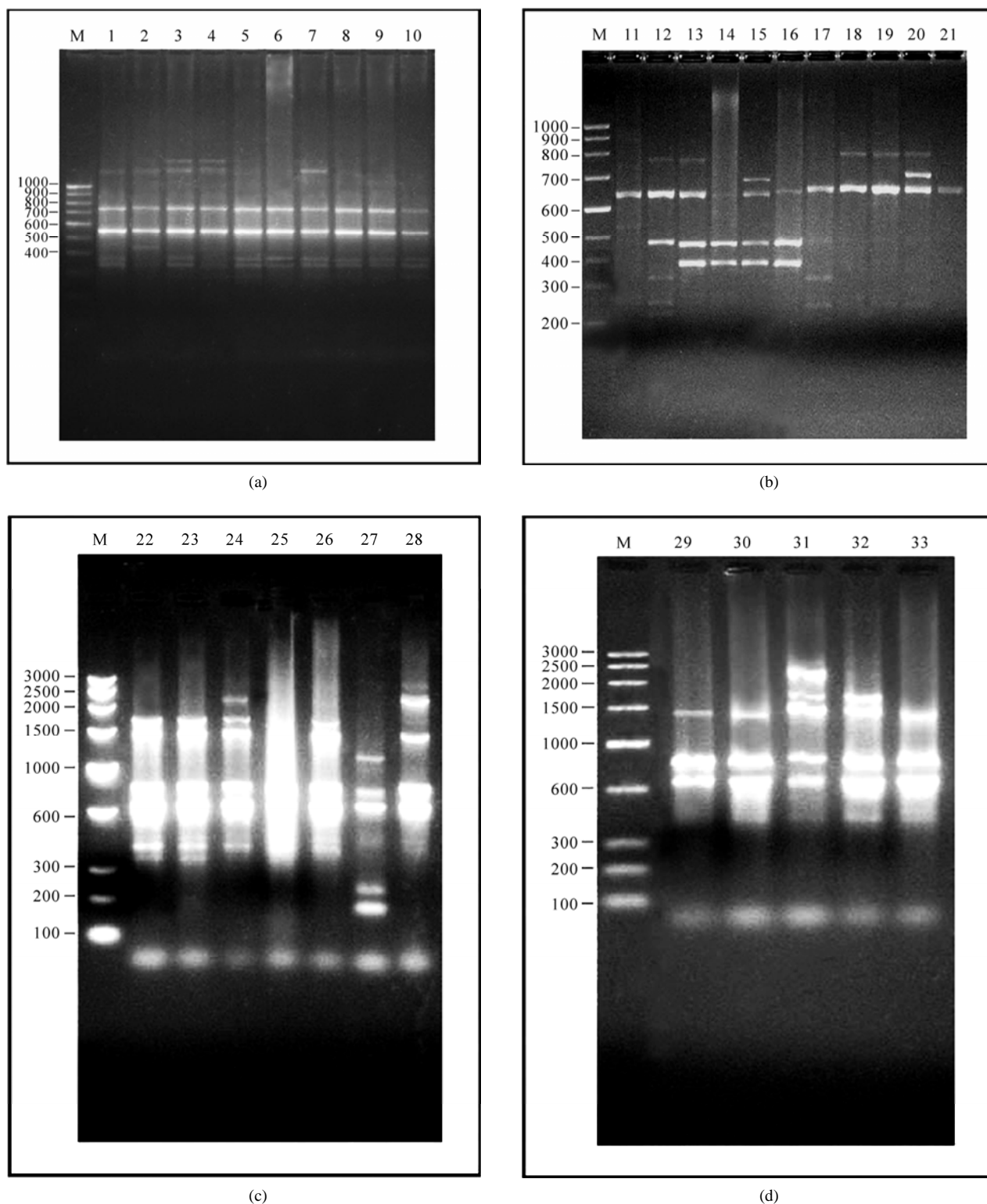
Figure 1. 12-23 SrRNA Spacer region amplicon of Gangatic isolates of *Salmonella*. (a) M: Marker 100 bp ladder (Baglore genei, India) Lane 1-10 *Salmonella* isolates (G29, G33, G36, G40, G23, G28, G35, G37, G38, G47); (b) M: Marker 100 bp (Baglore genei, India) Lane 11-21 *Salmonella* isolates (G4, G6, G7, G12, G13, G15, G17, G19, G22, G25, G39); (c) M: DNA Marker (100-3000) bp low range DNA rular (Baglore genei, India) Lane 22-28 *Salmonella* isolates (G1, G10, G14, G11, G16, G20, G21); (d) M: DNA Marker (100-3000) bp low range DNA rular (Baglore genei, India) Lane 29-33 *Salmonella* isolates (G45, G46, G50, G52, G55).

by 30 cycles composed of denaturation (94℃ for 1 min.), annealing (54℃ - 2min) and polymerization (72℃ - 2 min.). Final extension was carried out at 72℃ for 10 minutes. Five μl of amplified product was loaded on 1.5% agarose gel and electrophoresis was conducted in 1XTAE at 5V/cm. for two hours. Each sample was amplified thrice with negative control to avoid possibility of artifacts. Molecular weight of amplicon was measured by comparing 100bp ladder (Banglore Genei) using Bio1-D Software.

4. Results and Discussion

In all serovars, 16-23S r-RNA Spacer region was amplified. After separation of PCR product on 1.5% agarose gel only intense bands were scored. The relative molecular weights of amplicons were compared to the band with Standard molecular weight marker (100 bp ladder B.Genei India). Size of bands ranged from 144 to 2435 bp. All the isolates could be grouped into 15 profiles (M1-M15). M1 was most common and observed in 11 isolates. Eight profiles (M1, M8, M9, M10, M11, M12, M14 and M15Profile) were observed in 24 isolates of *Salmonella enteric* serovar Typhimurium. Two profiles

M2 and M3 were observed in four isolates of *S*.Abuja. Three isolates of *S*.Lagos exhibited three different profiles M4, M5 and M6. Two isolates of *S*.Chinkaul and one isolate of *S*. Oritamerin shared common profile M2.One isolate of *S*. Zwickau obtained from Narora had altogether different profile M7 (**Figure 1**, **Table 1**). Comparison of similar and dissimilar bands of amplicon with Jaccord's coefficient value revealed genetic variability in different isolates. On cluster analysis using Unweighted Pair Group Methods with Arithmetic Mean (UPGMA) the genetic similarity between 33 different isolates of Salmonella ranged from 0 to 100% and five major clusters were produced five major clusters at the level of zero similarity index.

In this study eight serovars of *Salmonella* were isolated by molecular methods from Ganges river water at six different locations (stations). *Salmonella enterica* serovar Typhimurium is considered a life threatening pathogen with zoonotic importance [14,15] and is found to be commonly associated with multiple drug resistance in human clinical cases [16]. *Salmonella* Oritamerin which is common in other countries [17] but not so frequently reported in India were present in Ganges water possibly due to the congregation of vast human population from across the diverse geographical boundries during
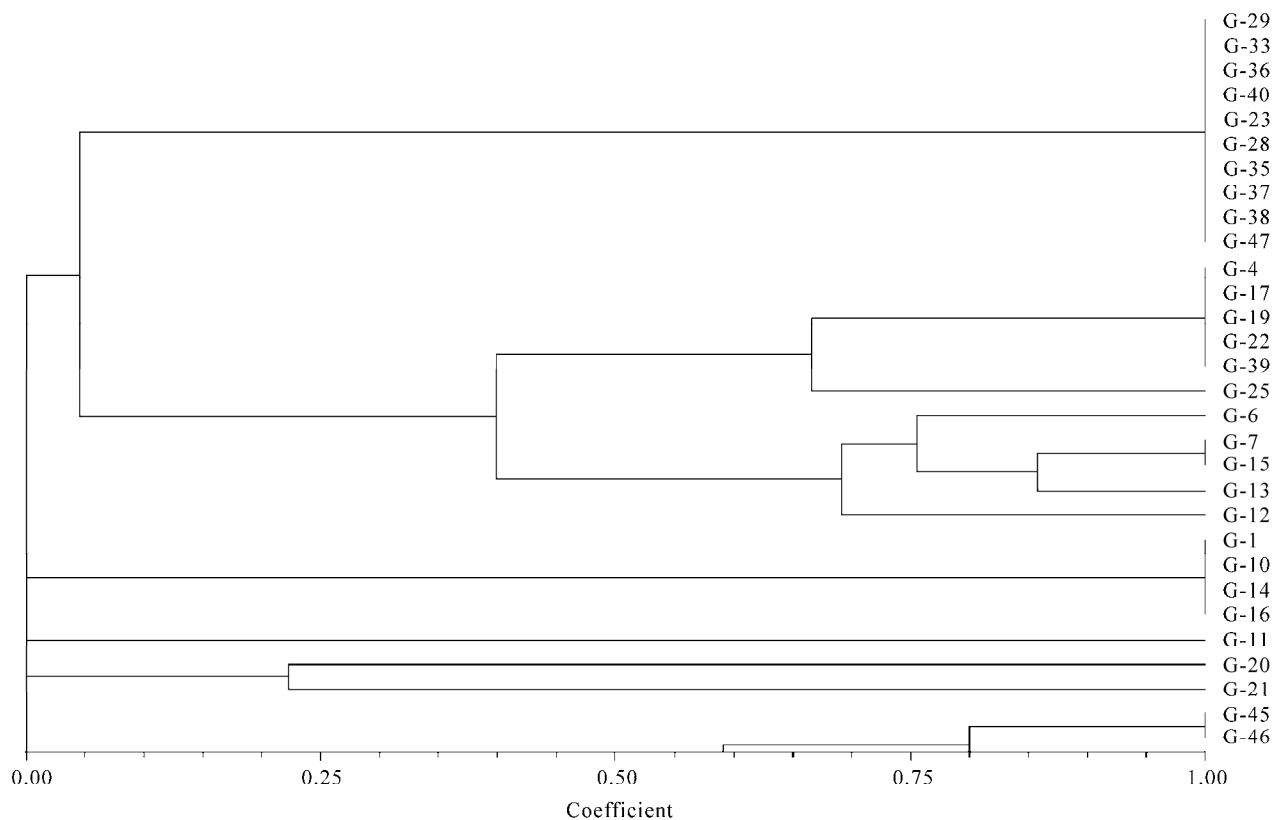
Figure 2. Dendrogram visualizing genetic variation in 16-23S rRNA spacer regions of isolates of *Salmonella* isolated from Ganges water.

festivals such as Kumbh.

On amplification of spacer region the product size varied from 529 to 2435 bp, which is very similar to the product size range reported by Jensen and Hubner [18]. The Variation of rRNA loci in *Salmonella* genome [19] and high genetic diversity of intergenic spacer region [20] could be attributed as possible reasons for high genetic variation shown by the isolates. We did not observed any serovar specific profile in the present study. *S.* Abuja and *S.* Chinkaul serovars shared the common profile M2 and *S,* Typhimurium the most common serovars observed in this study exhibited eight profiles. With these findings we suggest that 16-23S rRNA spacer region is not serovar specific but has a high genetic variation can be used for molecular typing of field isolates of *Salmonella*.

5. Acknowledgements

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Mercury Pollution from Dental Amalgam Waste in Trinidad and Tobago

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Abstract

Aim: To assess the knowledge and attitudes of dental practitioners regarding the disposal of amalgam waste from dental practices and to assess the level of mercury released via dental amalgam waste into the environment in Trinidad and Tobago. Method: A questionnaire on dental wastewater discharge and amalgam waste disposal was administered to dental practitioners in Trinidad and Tobago. Levels of mercury in samples of wastewater and solid deposits obtained during removal of 160 amalgam fillings, were measured using Atomic Absorption Spectrometry. The numbers of amalgam fillings placed and removed over a monthly period from a random sample of dental practices in Trinidad and Tobago were also obtained via the questionnaires. The amount of mercury entering the environment from dental practices was estimated from these data. Results: Twenty per cent responded to the questionnaire. Thirty per cent of respondents used elemental mercury (from a dispenser), while 74.4% used pre-capsulated mercury for preparing amalgam fillings. Seventy nine per cent used chair-side traps and filters but none had amalgam separators in their surgery. Methods used to dispose of amalgam waste included disposal in the trash (48.8%), washing down the sink (39.5%); and as hazardous waste (37.2%). A mean concentration of 0.0759 ppm (or mg/L) mercury was found in filtrate from the wastewater samples. A total concentration of 3.4 g mercury per dentist per day was found to be released into the environment via dental amalgam waste in Trinidad and Tobago. Conclusion: Best management practices for disposal of dental amalgam waste are not generally followed. At 3.4 gms per day per dentist, the level of mercury released via dental amalgam waste into the environment in Trinidad and Tobago may be too high. Dental practitioners require education on the management of dental amalgam waste and national legislation to protect the environment from this source of mercury may be required.

Keywords: Dental Amalgam, Mercury Pollution

1. Introduction

An amalgam is an alloy of Mercury and one or more other metals. The amalgam alloy is a combination of solid metals containing mainly particles of silver, tin and copper [1]. Other particles, which are sometimes included, are zinc, palladium, indium and selenium. Dental amalgam is made by mixing liquid mercury with powdered amalgam alloys in a process called "trituration". Trituration of commercial amalgam alloys produces a plastic mass (the "amalgam") that the dentist places in a cavity preparation [2].

Mercury occurs naturally in the environment and exists in several forms. It may change between different states and species in its cycle, but its simplest form is

elemental mercury, which itself is harmful to humans and the environment. Once mercury has been brought into circulation in the biosphere by human activity it does not disappear again in time spans comparable to human lifetime [3].When compared with industrial pollution and combustion of fossil fuels by vehicles, the mercury contained in dental amalgams is not a major source of mercury release to the environment, (globally, approximately 10,000 tons of mercury are mined each year, with an estimated 3-4% used in dentistry [4]. However, its environmental impact is mainly due to the poor management of dental amalgam waste.

During the 1970s and 1980s, 50% of the mercury used in dental practices was discharged into the environment: 20% of this was lost to the air and 30% to the sewage

system. During this period 5.6 tonnes of mercury per year were discharged into UK sewers. Furthermore, only 5% of the dental practices surveyed used amalgam separator in their waste disposal system [5]. Previous studies have established the level of mercury in the filtrate from wastewater discharged during amalgam removal to be approximately 0.1493 ppm (or mg/l) when no amalgam separator is used [6]. The total estimated release in one study was 1.196 g/day/dentist [7].

Evacuation systems in existing dental practices typically contain either one or two components that are capable of removing solids such as amalgam particles from wastewater-chairside traps and vacuum pump filters. Chairside traps are commonly used in both wet and dry vacuum systems. They remove particles down to approximately 0.7 mm. In addition to chairside traps, systems with a wet vacuum pump typically have a filter located just upstream of the pump that is designed to protect the pump from large solids. Vacuum pump filters have pore sizes of either 0.84 mm (20 mesh screen) or 0.42 mm (40 mesh screen). Chairside traps and vacuum pump filters successfully remove anywhere from 40-80% of the total mass of amalgam particles from dental office wastewater, leaving 20-60% to be discharged to sewers and septic systems [8].

Use of disinfectants containing oxidizing substances in dental aspirator kits may contribute to remobilization of mercury and its subsequent release into the environment [4] In addition, municipal wastewater treatment systems are not designed to treat hazardous waste or reduce mercury loadings to the environment. Consequently, all mercury in the influent wastewater remains unattenuated in municipal treatment plants, and either settles out in the grit chamber or residuals (sludge, or "biosolids"), or passes through the system to be discharged into a downstream lake, river or ocean along with the "treated" effluent. This discharge of mercury directly in the sewer system may greatly increases the possibility for the formation of the more bio-available organic mercury compounds.

In Trinidad and Tobago, no extensive research been conducted into the effect of mercury on our environment and people and no previous research has been done to estimate levels of mercury output from dental practices. There are currently over 235 registered dentists practicing the country and the Dental Association acknowledges that there are hundreds more unregistered persons who practice dentistry. Many of these practices and practitioners still use dental amalgam. At present no definitive legislation exists to regulate the use or disposal of dental amalgam in this country nor is there a company that deals with the disposal of collected amalgam waste. As such it is usually kept under water in a jar and then disposed of along with normal waste.

The objective of this study was to assess the knowledge and attitudes of dental practitioners regarding the disposal of amalgam waste from dental practices and to assess the level of mercury released via dental amalgam waste into the environment in Trinidad and Tobago.

2. Methods

Part 1–Questionnaire

A questionnaire discussing dental wastewater discharge, disposal of amalgam waste and use of filters and separators in dental chairs, was compiled and sent to dentists on the Dental Register in Trinidad and Tobago. The questionnaire also included questions on the number of amalgam fillings (restorations) removed on a monthly basis and the sizes of the fillings.

In order to obtain the level of mercury released via dental amalgam waste, an experiment had to be conducted utilising a typical dental chair unit as described in Part II. The percentage of amalgam entering the sewer system from the removal of dental amalgam from different sizes of fillings as well as the concentration of mercury in the wastewater entering the sewer system was calculated.

The data obtained was used to estimate the quantity of dental amalgam and potentially, the quantity of mercury released from dental practices into the environment. This was done by correlating experimental data from Part II with data collected via the questionnaire regarding the amount of amalgam fillings removed over a monthly period by dentists in Trinidad & Tobago.

Part 2–The aims of this part of the study were to determine the percentage of amalgam retained in solids separators and the amount lost to the sewerage system during the removal of dental amalgams, as well as to determine the concentration of mercury in the wastewater that enters the sewer system.

The collection of wastewater samples from a typical dental chair unit in a clinical environment and analysis of the amount of mercury present using Atomic Absorption Spectrometry were done by the following steps:

1) Anatomical replica teeth were prepared for and restored with 1, 2, 3 and 4 surface amalgam restorations. They were divided into four (4) groups each consisting of n = 40 and allowed to set for a period of 48 hours.

2) Before removal of restorations from each set of teeth, the dental unit was thoroughly flushed with water, and this sample collected was used as a blank. All restorations were removed with a Tungsten Carbide High Speed, bur in a water-cooled Star 430K high-speed handpiece (Star Dental, Lancaster, Philadelphia) attached to a dental unit with conventional suction system, in a procedure that mimicked normal operatory conditions. The suction line leading to the vacuum pump was interrupted and attached to a surgical collection unit. This ensured

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that all particles in the suction line (liquid and solid) were collected prior to reaching the vacuum pump, and the public wastewater system. No hand instruments were used to pry restorations from cavities, and care was taken to avoid removing tooth material with the bur.

3) All of the wastewater from each set was collected in 500 ml Nalgene containers. After the restorations had been removed from each set of teeth, the screen (pore size 1.0 mm) in the conventional chairside solids separator was removed and the particles of amalgam trapped were collected.

4) Calculations for dilution and digestion of the liquid portion of each sample were performed. (**Table 1**). Mercury was analyzed on the Varian 600 Atomic Absorption Spectrophotometer, using the VGA-77 vapor generating accessory (Varian Canada Inc., Mississauga, Ontario). The detection limit for these measurements was 0.0001 μg/l (Picture 16). Standards were prepared from certified 1000 µg/ml Mercury Stock Solution (Item Number AA34N-5) obtained from AccuStandard Inc., New Haven, USA.

5) Calculations for dilution and digestion of the solid portion of each sample were performed (**Table 1**). Approximately 0.1 g of each of the solid portions of the sample was weighed (Mettler-Toledo AG204 Analytical Balance, Switzerland, to a precision of 0.1 mg) and digested with 5% Nitric Acid for about 30 minutes, at 70°C. The resulting mixture was then quantitatively transferred to a 200 ml volumetric flask and then diluted with deionised water accordingly.

6) The concentration of mercury in each samples (1 surface, 2 surface, 3 surface and 4 surface) for both liquid and solid portions were obtained.

3. Results

Responses were obtained from 20% of the practitioners to the questionnaire discussing dental wastewater discharge and amalgam waste disposal. Over 80% of the respondents were from privately owned dental practices, whereas 18.6% were from public health centers and nearly 75% of the dentists were members of the Dental

Table 1. Calculations to show how dilution factors were determined.

LIQUID DILUTIONS:

$$\text{Dilution Factor (D)} = \frac{\text{Mercury } \mu\text{g/l} \times \text{Sample Volume (A) ml}}{\text{Volume Diluted To (B) ml} \times \text{Instrument Reading (E) ppd}}$$

$$\text{Mercury } \mu\text{g/l} = \text{Instrument Reading (E) ppd} \times \text{Dilution Factor (D)} \times \frac{\text{Volume Diluted To (B) ml}}{\text{Sample Volume (A) ml}}$$

$$5 \text{ ml of Sample} = 100 \text{ ml of Solution}$$

$$5 \text{ ml of Sample} = \frac{100}{5}$$

$$\text{Dilution Factor} = 20$$

SOLID DILUTIONS:

$$\text{Dilution Factor (D)} = \frac{\text{Mercury } \mu\text{g/kg} \times \text{Sample Volume (A) ml}}{\text{Volume Diluted To (B) ml} \times \text{Instrument Reading (E) ppb}}$$

$$\text{Mercury } \mu\text{g/l} = \text{Instrument Reading (E) ppb} \times \text{Dilution Factor (D)} \times \frac{\text{Volume Diluted To (B) ml}}{\text{Sample Volume (A) ml}}$$

$$\text{Mercury mg/kg} = \mu\text{g/kg} \times 10^{-3}$$

$$\text{Mercury \%} = \text{mg/kg} \times 10^{-4}$$

$$0.1 \text{ g of Sample} = 200 \text{ ml of Solution}$$

$$1 \text{ ml of Sample} = 200 \text{ ml of Solution}$$

$$2 \text{ ml of Sample} = 200 \text{ ml of Solution}$$

$$\text{Dilution Factor} = 200 \times \frac{400}{2} = 40000$$

Association. Some of the relevant information regarding the chairside traps, secondary filters and amalgam separators from the questionnaire is outlined in **Table 2**. 14% of the dentists used disposable chair side trap filters, while 86% used reusable chair side trap filters. The frequency with which they cleaned the traps varied as follows: daily (72.1%); weekly (25.6%); and monthly (2.3%).

They managed the waste from the chair side traps by: Recycling (4.7%); Washing down the sink (39.5%); Disposal in Trash (48.8%); Treating as Infectious (Biohazard) waste (32.6%); and as Hazardous waste (37.2%). The percentages do not add to 100% as they could have responded to more than one option. The responses of registered Dental Practitioners to the question of the method of disposal of amalgam waste from chairside traps are represented in **Figure 1**.

It was also interesting to note that 74.4% of respondents to the questionnaire used pre-capsulated mercury, 79.1% used chair side trap filters and 0% had amalgam separators in their surgery. The extracted teeth with mercury amalgam fillings were disposed of as follows: 9.3% claimed they placed it in a container for pick up by an amalgam recycler; 20.9% placed it in a red bag for disposal as medical waste; while 74.4% placed it in the trash. Information regarding disposal of extracted teeth, non-contact amalgam mixing scrap, empty amalgam capsules, chairside screens, under-sink traps and vacuum filters or screens obtained from the questionnaire is outlined in **Table 3**. It is noteworthy that in all instances the majority of practitioners dispose of amalgam waste in the trash. The data also revealed the average number of amalgam fillings removed per month by dentists who responded was 29.4.

For 1 Surface restorations, 19.95% of the total weight of the amalgam restoration removed from teeth was captured by the conventional chairside trap, whereas 80.05% of the total weight was not recovered. For 2 Surface teeth,

Table 2. Information concerning amalgam waste collection.

Use of chairside trap filters	Disposable trap filters	14%
	Reusable trap filters	86%
Frequency of cleaning traps	Daily	72.1%
	Weekly	25.6%
	Monthly	2.3%
Use of a secondary filter	Yes	83.7%
	No	16.7%
Possess amalgam separator	Yes	0%
	No	100%
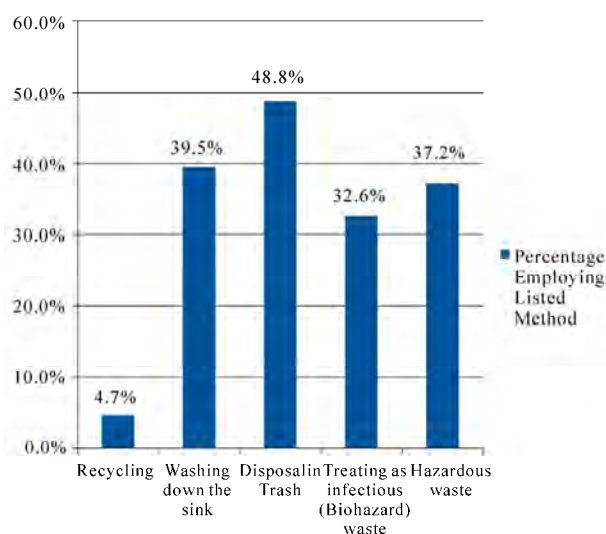
Figure 1. Methods used to dispose of amalgam from chairside traps.

Table 3. Information concerning amalgam waste disposal.

	Placed in a container for pick up by an amalgam recycler	Placed in a red bag for disposal a medical waste	Placed in the trash	Placed in the sink
Disposal of extracted teeth with amalgam fillings	9.3%	20.9%	74.4%	0%
Disposal of non contact amalgam mixing scrap	32.6%	23.3%	37.2%	2.3%
Disposal of empty amalgam capsules	2.3%	16.3%	67.4%	0%
Disposal of amalgam waste from chairside traps	14.0%	18.6%	51.2%	4.7%
Disposal of amalgam waste from under sink traps	11.6%	20.9%	48.8%	2.3%
Disposal of amalgam waste from vacuum filters	7.0%	18.6%	51.2%	2.3%

18.60% of the total weight of the amalgam restoration removed from teeth was captured by the conventional chairside trap, whereas 81.40% of the total weight was not recovered. For 3 Surface teeth, 12.81% of the total weight of the amalgam restoration removed from teeth was captured by the conventional chairside trap, whereas 87.19% of the total weight was not recovered. For 4 Surface restorations, 31.27% of the total weight of the amalgam restoration removed from teeth was captured by the conventional chairside trap, whereas 68.73% of the total weight was not recovered.

Therefore only 13%-30% of the weight of restorations removed, were recovered in the primary chairside trap and the bulk of the waste generated (70%-87%) was contained in the dental effluent waste.

On average, the concentrations of mercury in the liquid portion (filtrate) of the samples were highest in 4 Surface (0.1087 ppm (or mg/l)), followed by 2 Surface (0.0987 ppm (or mg/l)), 3 Surface (0.0697 ppm (or mg/l)) and 1 Surface (0.0266 ppm (or mg/l). The mean concentration of mercury in the liquid portion of all 4 sets of restorations combined (40 teeth) was 0.0758917 ppm. **Table 4** shows the results for the mean concentration of mercury in the filtrate of the various sizes of restorations removed chairside as well as the overall mean concentration of mercury in the filtrate.

The mean concentrations of mercury in the solid portion of the samples were highest in Surface 2 followed by Surface 3, Surface 1 and Surface 4. The mean concentration of mercury in the solid portion of all 4 sets of restorations combined (40 teeth) was 4077744.885 ppm (see **Table 5**).

Using the values obtained from chairside testing for mercury concentration in the liquid filtrate and solid portions as well as data from the dental wastewater discharge questionnaire, the total mercury discharge into the environment from removal of dental amalgam restorations was estimated (**Table 6**).The combined concentration of mercury released by all dentists in Trinidad and Tobago, on a monthly basis was 22285.35 g/month. It was estimated that the combined concentration of mercury from both solid and liquid portions released from

Table 4. Mean concentration of mercury in filtrate from samples.

No. Of Restored Tooth Surfaces	Mean Concentration of Mercury In Filtrate ppm (or mg/l)
I	0.0266
II	0.0987
III	0.0697
IV	0.1087

Mean concentration of mercury in filtrate = 0.0759 ppm

Table 5. Mean concentration of mercury in amalgam deposits from samples.

No. Of Restored Tooth Surfaces	SOLID DEPOSITS CONCENTRATION (ppm)
1-SURFACE	3705180.702
2-SURFACE	4848732.847
3-SURFACE	4690376.106
4-SURFACE	3066689.882
MEAN CONCENTRATION	4077744.885

Table 6. Estimated average mercury discharge from amalgam restoration removal.

Estimated Average Combined Concentration of Mercury For All Dentists for 1 month	22285.35 g/month
Estimated Average Combined Concentration of Mercury per Dentist per month	94.82 g/month
Estimated Average Combined Concentration of Mercury per Dentist per day	3.4 g/day

the removal of dental amalgam was 3.4 g per dentist per day.

4. Discussion

Dental amalgam, in widespread use for over 150 years, is one of the oldest materials in oral health care. Dentists in the United States placed about 71 million amalgam restorations in 1999 [9]. The history of the controversy surrounding amalgam use is well documented [10]. Whereas the debate on the health effects of amalgam use is well highlighted, the possible impact of mercury from dental amalgam entering the environment has received much less attention. Though Dental Amalgam is a highly favoured restorative material, there is controversy mainly because of human health and environmental concerns.

All dentists on the dental register in Trinidad and Tobago were sent questionnaires however the response rate in this study was approximately 20%, which is low but comparable to other studies with 12% [11] and 28% [12]. Also as the dentists were asked to estimate the number of amalgam restorations removed monthly the possibility of recall bias may have occurred.

A study found that more than three-quarters of King County, Washington, dental offices did *not* reclaim or sequester mercury bearing waste captured in chair side traps or pump filters [13]. Rather, they put it in the garbage, mixed it with medical waste, stored it onsite or put down the drain. These findings were similar in this study with the majority of dental offices placing the waste from

the traps and filters into the trash or washing it down the sink.

There is enormous variation in the size and shape of waste amalgam debris. The bulk portions are irregularly shaped pieces of amalgam that break away during the process of removing the amalgam restoration from the tooth. They may be up to several millimetres at the widest dimension. Small particles produced from contact with burs operated by an air turbine hand-piece take the form of chips and filings varying in size from a few microns to sub-micron dimensions. Normally, water, saliva, and waste amalgam are removed from the operating site and oral cavity by means of high volume suction.

The bulk portions are relatively easy to capture in the chair-side solids separator (primary trap) because of their substantial size. Virtually all the particles of micron and sub-micron dimensions produced by contact with the bur pass through the primary trap and enter the secondary solids separator (secondary trap) associated with the vacuum pump. Here some settle to the bottom of the trap, but turbulence of water passing through the trap carries the remainder into the waste stream [14].

In part II of the study, samples of wastewater were collected from the discharge pipe, which had been completely disconnected from the drain, during the removal of dental amalgam restorations. This method of sample collection minimizes opportunities for particles to settle out of suspension in the wastewater stream as will occur if a sampling valve is inserted into a wastewater line downstream from the pump. Also, if only a part of the wastewater is collected through a sampling valve the amount of particles in the discharged wastewater will not be accurate because particles of amalgam are not distributed uniformly in the wastewater stream. Furthermore, if the vacuum system is shut down while a sample is taken many particles of amalgam will settle out of suspension. Therefore, this sampling technique ensured that not only dissolved mercury was obtained, but also all the particles of amalgam in the wastewater. A high volume vacuum pump system that requires a continuous flow of water to maintain a seal with the impeller and maintain vacuum was also used.

In order to conserve water some pumps re-circulate a percentage of the water used to maintain the seal. However, the basic principle of operation and solids collection is similar between different brands of these pumps. Still, our ability to recover 31% or less as solid waste in solids separators may be biased because only one high volume vacuum pump system was used in this study. A lower or higher rate of water consumption by different brands of pumps, or by different water flow adjustments, may influence the concentration of amalgam particles and dissolved mercury as a consequence of the dilution factor.

A higher concentration of mercury was found in the amalgam deposits from the wastewater (the effluent),

whereas only a relatively minute quantity of the mercury was dissolved in solution (filtrate from the wastewater samples). This was because of the differences in the dilution factors; the dilution factor for the liquid portion of the samples was = 20, whereas the dilution factor for the solid portion of the samples was = 40000. Hence there were more particles of mercury per individual solid sample, as there were per individual liquid sample. In this experiment the wastewater contained about 70% to 87 % by weight of the waste generated during the removal of amalgam restorations. This is consistent with previous research that reported that less than 0.3% of amalgam waste is soluble [4].

Previous studies have shown a wide variation in the level of mercury in the filtrate from wastewater discharged during amalgam removal, from 0.1493 mg/l (ppm) when no amalgam separator is used [6] to 9.7-306 mg/l (ppm) [15].This study found the level of concentration of mercury in the filtrate to be 0.0759 mg/l (ppm) without the use of an amalgam separator. Since these were experiments, the high range is expected because of the large number of variables associated with experimentation. A fraction of particles would have also been lost in the patient's mouth and the operating environment during the removal of restorations

The estimated release of mercury in dental amalgam has been previously reported to be 1.196 g/day/dentist [7], 250 mg of mercury/day/dentist [16] and 0.46 g-271 g daily per dentist [17]. In Canada, other studies have estimated release of 125 mg per dentist per day of mercury into waste water annually [18], 131 mg/dentist/day [19] and 172.7 mg per dentist/day in Ontario if no amalgam separators were used [20]. The current study estimated the release of mercury from dental amalgam to be 3.4 g/day/dentist.

Zero percent (0%) of the dentists reported using any amalgam separating device. The use of ISO-certified amalgam particle separators by all dentists could dramatically reduce dentistry's share of mercury in Trinidad and Tobago's municipal sewage treatment plants. If the amalgam waste generated is not captured before it leaves the dental surgery, it will enter the wastewater system, the sewers and eventually the rivers and oceans. If it is captured but improperly disposed of, it poses an environmental hazard particularly because of the mercury content.

Although lower levels of mercury were found in the filtrate in our study, significantly higher overall levels were estimated as being released into the environment. This may be due to the fact that in estimating the total mercury release the amount of mercury in the amalgam solids in the chairside trap as well as the waste water stream were combined. Additionally, amalgam is still a highly favored restorative material in Trinidad and Tobago. This means that with more amalgam being used and 0% of dentists using amalgam separators, higher

levels of mercury may be entering the environment via the wastewater stream. Also with no company possessing the capability of recycling waste amalgam in the country, any amalgam collected either via separators or chairside filters and traps will eventually enter the environment either via landfills or incineration.

Mercury levels in our marine environment are elevated [21,22]. No prior work existed to determine how significant a contributor dental amalgam is to these levels. In Trinidad and Tobago, failure to enact the Water Pollution Rules and the Air Pollution Rules along with other pieces of important environmental legislation, has proven to be a hurdle in the regulation of dental amalgam waste.

The current situation however means that this country is ideally positioned for an in depth amalgam waste disposal policy to be properly formulated and implemented in conjunction with appropriate education of practitioners and their staff on the need for careful management of dental amalgam wastes. This research may therefore pave the way for future detailed testing as well as the design and implementation of appropriate policy in Trinidad and Tobago.

5. Conclusions

Although dental practitioners in Trinidad and Tobago may have some information about methods of disposal of amalgam waste, education on these methods is required. In addition, best management practices though easy to implement are not often followed. At 3.4 g/day/dentist the level of mercury released via dental amalgam waste into the environment in Trinidad and Tobago may be too high. Separators in dental practices and the incorporation of a company to effectively recycle or process collected waste amalgam, may help control the discharge of amalgam into the wastewater stream.

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