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RESEARCH ARTICLE

Vermitechnology – an Eco-biological Tool for Management of Solid Wastes, with Special Reference to Pressmud, Sludge and Cowdung.

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ABSTRACT

The solid waste management by using earthworm *Eudrilus eugeniae* in three types of wastes (sludge, pressmud and cowdung) was investigated. Among the three vermicompost produced cowdung compost is more effect in growth of agricultural crops, the composition of micronutrient was higher in vermicompost when compared with normal compost.

Key words: Solid waste, vermicompost, micronutrients.

INTRODUCTION

India produces about 3 million tonnes of organic wastes annually which could be utilized for recovering important resources like fertilizer,fuel wood and fodder. This huge amount of waste has also the potential to produce 4 million tonnes of plant nutrients besides biogas and alcohol. Dash and Senapati, (1985), Bhattacharjee (2002) and Mitchell (1978), reported that earthworm is physically an aerator, crusher, mixture and chemically degrader and biologically stimulator in decompose system. Microbial composting of organic wastes through earthworm activity is called vermicomposting, which did not involve the thermophilic stages. Products of vermicomposting are organic fertilizer, called vermicompost and earthworm biomass that forms vermiprotein Dominguez et al., (1997) reported that

vermicompost is a peat like material with excellent structure, porosity, aeration, drainage and moisture holding capacity. Vermicomposting among other alternatives has been considered as way to transform wastes into useful component for plant and soil diminishing the negative environmental input. Organic manure containing essential amino acids increase the chlorophyll content of leaf which in turn enhances metabolite synthesis resulting in crop productivity [2]. The worm castings are good fertilizer additive for agricultural crops. Purakayastha and Bhatnagar (1997) reported that vermicompost besides being a source of essential plant nutrients, vitamins and growth hormones, is also known to posses antagonistic action against bacteria and fungi.

Buchanan et al., (1998)[1] reported that vermicompost have higher level of available nutrients from which they were formed. Humic acid is a very important constituent of vermicompost. Many of its important properties are slow release of plant nutrients improvement of soil physical properties enhancement of micronutrient of plants through chelation, reactions etc [7]. Plant growth regulation belonging to the auxin, gibberellins and cytokinin groups present in the worm worked material were produced by a wide range of soil microorganism. Many of which live in the guts of earthworm or with in the castings [11].

Lund and Jacobson (1994)[6] reported that there is much more nitrogen in worm cast than in parent soil, more over cast as higher base exchange capacity and more exchangeable calcium, magnesium, potassium and available phosphorous than the soil in which the worm live. It is inevitable that the biological methods in land reclamation provide food possibilities for upgradation or conservation of soil fertility on sustainable basis. Due to close coupling of earthworms to process and functions of ecosystems, land reclamation by earthworm is an exiting approach to improve the degraded lands in various parts of the globe.

MATERIALS AND METHODS

Earthworms were collected from the Sathyamangalam farm, Erode district and the Earthworm Rearing Centre, Greenieva, Vadavalli, Coimbatore. The worms were maintained under normal weather conditions. The industrial waste viz. alcohol sludge was collected and transported to the working unit from the distillery factory, Udumalpet. The bulking agent saw-dust was collected from Ganesh saw mills, Vellakinarpirivu, Thudiyalur, Coimbatore. The sludge since freshly collected had high water content to reduce the excess heat and volatile matter. The heaped sludge was well spread and sun dried. The cow dung was powdered and given to the earthworm and kept as control in the second sample sludge mixed with sawdust 2:4 Kg and sawdust mixed with sludge 4:2, sawdust mixed with sludge 3:3 Kg. Third sample pressmud mixed with sawdust 2:4 kg and the sawdust mixed with pressmud is 4:2 and the sawdust mixed with pressmud as 3:3 Kg. These composts were applied to the agricultural crops and growth rate was recorded.

Materials for vermicomposting

Agricultural residues require minimum of two to three weeks for earthworms to accept them as substrate and feed. Among them, sericulture wastes require minimum forty days for earthworms to accept this material as feed. This is because of chemicals used in the silkworm rearing beds and also due to uric acid and ammonia levels in excreta of silkworms. On increasing the time of primary decomposition, material can be used for vermicomposting. The recovered vermicompost will have high levels of plant organic matter [5]. In this organic matter, heat generation will be high and persist for long time. If this is mixed with other biomass and cow dung, the material becomes palatable for earthworms in forty days.

Agro industries like paper mills, sugar factories and alcohol distilleries release effluents that will have high levels of nutrients required for plants. These effluents when mixed with solid matter like coir pith and sawdust, can become the source of feed for earthworms to get vermicompost as final product. The simple technology of using suitable earthworms and mixing these materials with other organic matter made them acceptable for earthworms to bring down the level of environmental pollution.

It is essential to identify the parameters to determine the maturity of vermicompost that will assure the safety measures for its use as good soil amendment. If this point is over looked, then some of the products may even turn deleterious to soil and crops. Vermicompost produce in a proper manner must have certain physico-chemical, biological and biochemical characteristics.

RESULTS

The physical parameter colour of the compost is brownish, colour of the sludge is dark brown and the colour of pressmud is brownish. The electrical conductivity values are more in the sludge the minimum electrical conductivity value was recorded in the compost. A moderate conductivity value was recorded in the pressmud , appearance of compost is cylindrical in shape, sludge is pellete and pressmud is small stick like appearance.

pH of the compost was 7.2, the pH value of sludge was 7.4 and the pH value of pressmud was 7.6.The phosphate value of compost was 2.42, the phosphate value of sludge was 6.6 and the phosphate value of pressmud was 2.048 mg/l. The value of nitrate was recorded as maximum in the sludge (714.08 mg/l) and the minimum value of nitrate was recorded as (101.93 mg/l) in the compost. The nitrate value of pressmud was 700.40 mg/l.

The minimum silicate value was recorded as 3.31 mg/l in the pressmud, the maximum value of silicate was recorded as 22.12 mg/l in the sludge. The silicate value was recorded as 78.31 mg/l in the compost. The value of iron shows minimum in the pressmud (0.13 mg/l) and maximum in the sludge (6.62 mg/l). The value of compost is zero. The minimum value was recorded as 195 mg/l in the compost and maximum value was 485mg/l in the pressmud and the sludge showed the value of 200mg/l. The minimum chloride value was noted in the compost as 269.80mg/l, the maximum chloride value was noted in sludge as 2832.90mg/l. The chloride value of pressmud was 2016.40mg/l.The minimum and maximum value of sulphate was recorded as 3.84mg/l and 21.12 mg/l in the compost and sludge respectively .The pressmud showed the value of 11.52 mg/l.

The pressmud mixed with sawdust slowly converted into compost. In cow dung the conversion of compost was faster. The breeding and multiplication of earthworms are more in the cow dung samples, whereas slow in the pressmud and the sludge samples. In field compost from cow dung, sludge and pressmud applied to banana, pumpkin,beans,tomato,brinjal and bitter guard. The flower, pod, production is more in the cow dung compost than in the pressmud and sludge. Minimum flower and pod formation obtained from the pressmud and sludge composts. Plant disease (infection by scale insects) also observed in the plant reared with sludge and pressmud composts. Weight of the vegetables are more in the cow dung compost than in the sludge and pressmud composts.

In the present study, the earthworm *Eudrilus eugeniae* the African varieties were collected from the two centers, one from Sathyamangalam, Erode and another one is from Greenieva,Vadavalli,Coimbatore. The reason for collection of exotic varieties was it has more efficient in the compost production than the Indian varieties.

The solid materials were collected for solid waste management studies they are cow dung, distillery sludge and sugar factory pressmud. Cow dung was collected in the kasinanjekoundanpudur located one kilometer away from

the college. The sludge was collected from the distillery factory located at Amaravathy nagar, Udumalpet, Tiruppur district.The pressmud was collected from the Amaravathy sugar factory located at Udumalpet, Tiruppur district. The earthworm was introduced into the above said three samples for the conversion of waste in to useful compost.

DISCUSSION

The colour of three samples namely cow dung, pressmud and sludge are due to various enzymes present in the body of earthworm and soil microbial fauna and flora, crushing of machine and other chemical processes during course of distillation.

The value of electrical conductivity was maximum in sludge and minimum in cow dung may be due to various chemicals used in sugar factory and nature of food intake of cattle. The pH value of compost was recorded as 7.2 it is very close to neutral condition. The pH value of pressmud was recorded as 7.6. This may be due to the nature of the solid wastes processed in the sugar factory and is alkaline condition. pH value of sludge was recorded as 7.4. It is also alkaline condition.

The maximum value of calcium, magnesium, phosphates, nitrates silicates, chlorides iron are recorded as maximum in the sludge. This might due to the nature of the distillery factory effluent. The value of calcium, magnesium, phosphates, nitrates silicates, chlorides iron are recorded as more in the pressmud than in the compost. But the values are very low compared to sludge. The value of iron was recorded as zero in the compost. This may be due to the nature of food taken by the cattle.

Various NGOs may play a vital role in managing solid wastes. They should be given judicial power by slapping fines on litterbugs. Awareness should be developed against littering. Enforcement of laws should be strict. The Delhi high court has recently directed MCD appropriate places. Such measured areas are very effective and will make the differences, provided they are properly implemented.

Earthworms have very few mechanisms to preserve their body moisture. Their moist body surface requires moist environment without which they perish in short time. Optimum cocoon production occurs between 28 and 42 per cent soil moisture [4] and population densities are usually the highest between 12 and 30 per cent soil moisture [8]. In practice this means keeping the earthworm do not thrive well in dry soils or during drought seasons and heavy flooding. Moisture also governs the growth, maturation and cocoon production of worms [12].

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Table 1: Shows the physico-chemical parameters of the experimental samples (Values expressed as mg/l except pH)

Name of the sample	Colour	E.C	Ca	Mg	NO_3	SiO_3	SO_4	Cl	Fe	pH
Cow dung	Brownish	0.05mMhos	70	195	101.13	3.31	3.811	269.8	0	7.2
Press mud	Dark brown	0.29mMhos	115	485	700.40	18.31	11.52	2016.40	0.13	7.6
Sludge	Brownish	1.9mMhos	750	200	714.08	22.12	21.12	2832.90	6.62	7.4

Table 2: Shows the physico-chemical composition of vermicompost (Vermicompost analyzed, produced from different organic wastes as a raw material, with 25% dung as a necessary ingredient. Source: NORA, Pune)

Bulk density	0.5 to 0.6 g/ cc
Water-holding capacity	150 to 200 %
Pore space	40 to 60%
Moisture	20 to 25%
Colour	Black /Brown
Texture	Non sticky granular
Organic Carbon	8.00 to 22%
Total Nitrogen	0.5 to 1.5%
Available Phosphorous	0.3 to 0.5%
Available Sodium	500 to 600 ppm
Calcium and Magnesium	20 to 75 Meq/ 100 g
Copper	2.0 to 23.0 ppm
Iron	2.0 to 10.0ppm
Zinc	5.0 to 15.0 ppm
Available Sulphur	100 to 66 ppm
Available Potassium	0.25 to 0.6%

Table 3: Characteristics used for distinguishing different ecological types of earthworms (Bouche,1977)

Character	**Ecological type**		
	Epigeics	**Anecics**	**Endogeics**
Body	Small	Moderate	Large
Burrowing	Reduced	Strongly developed	Developed
Longitudinal contraction	Absent	Present	Least developed
Hooked chetae	Absent	Present	Absent
Sensitivity to light	Feeble	Moderate	Strong
Mobility	Rapid	Moderate	Feeble
Skin moistening	Developed	Developed	Feeble
Pigmentation	Homochronic	Dorsal and anterior	Absent
Fecundity	High	Moderate	Limited
Maturation	Rapid	Moderate	Slow
Respiration	High	Moderate	Feeble
Survival	As cocoons	True diapause	By quiescence

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Study on Hepatoprotective Effect of Heart Wood Extract of *Pterocarpus marsupium* Roxb., in MSG-Induced Obese Male Albino-Mice (*Mus musculus*).

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ABSTRACT

The present study concerned with biochemical and histopathological changes in MSG-induced obese male albino mice, *Mus musculus* and highlighted on the possible Hepatoprotective effect of *P.marsupium* heartwood extract. Serum AST, ALT, ALP activities and lipid peroxidation were analysed in the experimental animals for ninety days of duration. After 90th day, histological study was carried out in control, MSG obese and extract fed obese groups. The results showed marked and gradual increase in serum enzyme markers (AST, ALT,ALP) and also in MDA level, an indicator of LPO. The significant increase was sustained in obese animals till the end of study period. Administration of PMS in obese mice for 90 days caused decrease of serum marker enzymes along with MDA and the effect was in a time-dependent manner. Histopathological changes noticed indicated obesity associated hepatic injury by MSG and on the otherhand, the treatment of heartwood extract was able to restore the hepato cellular damage by inhibiting lipidperoxidation and reducing oxidative stress linked to obese status in MSG obese mice. The hepato protective potential was exhibited through the phytoconstituents in the extract.

Key words: *Pterocarpus marsupium*, heartwood extract, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphate, lipid peroxidation, malondialdehyde.

INTRODUCTION

Obesity is one of the growing epidemic associated with many complications like hyperglycemia, hyperlipidemia, cardio vascular diseases, insulin resistances, neurodegenaration and some types of cancer[1,2].One of the contributors for the development of early and late onset of obesity among population in developed countries is the higher consumption of food additive and flavor enhancer Monosodium glutamate (MSG) which is used in all the packed and readymade food items[3]. MSG consumption even in lower doses may have deleterious effects with the resultant increase in body weight has been reported in animal and human studies [4-9]. MSG induced adverse effects on liver[10,11,12], kidney[13], heart[14], brain[15,16] and other tissues in MSG rodents are evidenced. Further studies reveal that MSG induced hepatotoxicity accompanied with lipid peroxidation due to reactive oxygen species (ROS) causing membrane damage and cellular integradity result in oxidative stress [10-12].

From ancient time natural antioxidants from medicinal plants have been reported to have therapeutic potential in health disorders. Pterocarpus marsupium (Indian Kino tree) has been used for treating diabetes, metabolic syndromes, hyperlipidemia, reducing body weight in clinical trials and many growing studies on diabetes induced animal models are reported. Due to the presence of flavonoids, the phenolic compounds,, alkaloids ,flavonoids, glycosides, terpenoids, tannins, phenols, saponins and steroids[17], each of the phyto chemical constituent seem to contain various properties like anti diabetic[18-20], anti peroxidative[21], anti hyperlipidemic[22,23] , anti inflammatory[17], cardio tonic and anti obesity effect[24]. Additionally the hepatoprotective effect of PMS extract on CCl_4 [20] induced liver damage and STZ-diabetic rats [23]. Based on earlier studies highlighted on the adverse effects of MSG inducing obesity, the study was designed to investigate possible effect of heartwood extract of *Pterocarpus marsupium* (PMS) on hepatic functions in MSG induced obese mice.

MATERIALS AND METHODS

Animals: Forty healthy adult male and female Swiss-strain albino mice (*Mus musculus*)w eighing between 20 and 25g were obtained from Kings Institute, Chennai. The mice were housed in animal house with exposure to 10–12 hr of daylight at a relative humidity of 30–70%. They were fed standard mouse feed and were given water *ad libitum*. After the animals bred, the new born male pups were used for the study. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee.

Chemicals: Monosodium glutamate **(MSG)** was purchased from the commercial shop which was labeled "Ajino-moto". All chemical reagents were of analytical grades and purchased from Hi-Media (Mumbai).

Plant material: The heartwood of *Pterocarpus marsupium* (PMS) was collected from Idukki district in Kerala. PMS heartwood was washed with distilled water, shade dried, powdered and stored in an air- tight container.

Preparation of extract: The heartwood of PMS (10g) was cut in to small slices, powdered and it was soaked overnight in distilled water (100 ml), boiled and filtered. The dosage used was 0.25 ml of 10% aqueous extract as fixed by Farzana [25].

Induction of Obese- diabetes mellitus in male neonatal mice: Subcutaneous injection of MSG (4mg/gm.b.wt) was given to the five days old neonatal pups on alternative days, each received six doses of MSG. The male mice were tested for diabetes from fourth week after the last dose of MSG injection by measuring blood glucose level. Animals of each group were weighed weekly. Development of obesity was confirmed by body mass index calculation at third month of the study period.

Experimental design: *Exposure:* The mice were divided into four groups each with 10 to 15 pups.

Group I : Served as control (male pups) which had 0.5% saline
Group II : MSG obese control-Male pups injected subcutaneously with MSG (4 mg/gm.b.wt.)
Group III : MSG obese male mice fed with crude extract of *P.marsupium* heartwood administered orally (0.25 ml / gm.b.wt /day) with a baby oral feeding tube.

Sample collection

At the end of 3rd month after the last MSG dose, the animals were fasted overnight and sacrificed by cervical dislocation. The blood was taken by heart puncture and centrifuged at 3000 rpm for 10 min to obtain the serum and liver was removed, washed in ice- cold physiological saline (0.9%), blotted and weighed. 100 mg of each tissue was homogenized in 0.1M Tris- Hcl buffer (pH 7.4) at 4ºC in a homogenizer. The homogenate was centrifuged at 3000 rpm for 30 min at 4ºC in a refrigerated centrifuge to obtain the supernatant and it was used for the estimation of biochemical parameters .such as malondialdehyde (MDA) an index of lipid peroxidation.

Biochemical Parameters

Lipid peroxidation: Lipid peroxides expressed as Malondialdehyde (MDA) was estimated using thiobarbituric acid reagent as described by Ohkawa *et al.,*[26].

Serum Hepatospecific Markers

Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel[27]. 0.05 ml of serum with 25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α - keto glutarate for SGPT, in phosphate buffer pH 7.4) was incubated for an hour in case of SGOT and 30 min. for SGPT. 0.25 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 505 nm in *uv-vis* spectrophotometer. Activities were expressed as U/L.

Based on the method of King and Armstrong [28]alkaline phosphatase activity was assayed using disodium phenyl phosphate as substrate. The colour developed was read at 510 nm in *uv-vis* spectrophotometer after 10 min. Activities of ALP was expressed as IU/L.

Histopathological Studies

Small pieces of liver tissue in each group were collected in 10% neutral buffered formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5- 6μm in thickness were cut and stained with hematoxylin and eosin (H&E). These sections were examined photo microscopically for histopathological studies.

Statistical Analysis

Data are expressed as mean ± S.D. Statistical differences were determined by the Student's *t*-test was calculated and the values were considered significant if *P*-values were less than 0.05($P<0.05$). Number of animals used was 10-15.

RESULTS

The activities of the serum enzyme markers of hepatic injury –SGPT/AST, SGOT/ALT, ALP are summarized in table and figure 1-3.Significant ($P<0.05$) elevation in the serum alanine amino transferase (ALT) and aspartate amino transferase (AST) and alkaline phosphatase (ALP) enzyme activities were observed in the MSG induced obese mice compared to the control groups. The increase in the serum AST activity was by 15.5%, 71.3% and 75.6% respectively

in the MSG obese animals from 0- 90 days (table-1). In the heartwood extract fed obese mice there had been significant (P<0.05) reduction in AST activity with the increase of duration of the extract administration and the reduction was by 5.2%, 10.8% and 13.4% after 30, 60 and 90 days of extract treatment.Table and figure -2 indicates significantly (P<0.0.5) increased ALT activity in MSG obese mice from 0-90 days as compared to the control mice and the increase was by 4.4%, 15.2% and 21%. In extract treated obese mice the decrease in ALT activity observed was by 21.2%, 23% and 33.5% after 30, 60 and 90 days of extract administration. The notable decrease was in a time-dependent manner.

The data shown in table and figure-3 revealed significant (P<0.05) increase of ALP activity in the MSG obese mice as compared to the control mice. The obese animals exhibited 22%, 78.3% and 86.4% increase at 30, 60 and 90 days old respectively. However, the extract treatment in the MSG obese groups could significantly (P<0.05) bring down the ALP activity by 8.7%, 19.3% and 42.9% after 30, 60 and 90 days administration respectively. A notable observation in the above mentioned enzyme activities (AST, ALT, ALP) is that there had been increased activities among control groups also from 0-90 days. The data shown in table and figure-4 represent MDA level in liver tissue and it revealed that there was more than 2 fold increase (113.13%) in liver at 0 day in MSG –obese mice as compared to its related control and this increase of MDA was 78.3% at 90th day in MSG-obese animals. There was also slight increase of MDA level from 0-90 days old control animal itself. Meanwhile, the PMS extract fed obese animals showed significant (P<0.05) reduction in MDA level as compared to MSG –obese mice. Where the reduction was initially by 29.7%, 36.2% and finally 48.6 % after 30, 60, and 90 days of extract administration.

Histological study

The control sections of liver showed normal histological features (Photos -1a &1b)with hepatocytes, central vein and sinusoids whereas in MSG obese mice dilated central vein, nuclear necrosis, degenerating changes in hepatocytes , nuclear swelling, pyknotic nuclei and loss of normal hepatic architecture with inflammation was observed.(Photos 2a&2b). The PMS extract administration for three months in MSG obese mice could restore the liver architecture with mild distortions in hepatocytes and sinusoids. (Photos - 3a & 3b).

DISCUSSION

In confirmation with previous investigations [1,3,4] MSG induced obesity by neo- natal administration was observed by 3rd month in male albino mice . In the present study, liver damage induced by MSG obesity was indicated from both biochemical and histological examinations. The extent of hepatotoxic effect was accessed by the levels of released cytoplasmic enzymes such as AST, ALT and ALP in circulation. Data from the study revealed significant increases in the activities of serum marker enzymes in MSG obese animals as compared to normal control group(Table 1-3) indicates hepatic injury taken place in obese mice induced. The increase of ALT and AST and ALP enzymes are a sensitive marker of liver damage. The observed enhanced activities of these serum markers imply that MSG could dissociate easily and spike blood plasma levels of glutamate which lead to excitotoxicity [29, 30]. Further, it is suggested that glutamate toxicity is more implicated in organs of higher metabolic activity in the animals [31]. Earlier reports indicate that ALT is a strong positive indicator of insulin resistance, obesity and diabetes mellitus[32]. Studies[31] shown that the propable increase in liver marker enzymes could be due to deamination of glutamate producing ammonium ions which becomes toxic to the animals needs to be detoxified via reactions of urea cycle. Hence, the liver when overloaded with ammonium ions due to the accumulation of glutamate causing imbalanced Ca2+ levels might result in damage to the liver, consequently releasing the serum marker enzymes. Additionally [34] has shown that high levels of excitotoxins cause damage to the brain which may lead to the increase in AST activity

which is also in accordance with the present results. Recent reports [33] indicate that elevated serum AST activity may be indicative of myocardial infarction.Further, increased ALP activity found in obese animals may be an indication of possible liver diseases associated with obesity. This results further agreed with those obtained by earlier investigators [11,12,14,35,36,37] suggesting the possibility of cirrhosis.

In addition, the increased ROS formations causing enhanced lipid peroxidation revealed by the MDA level current study(Table 4) denotes the accumulation of glutamate toxicity and more Ca2+ influx leading to mitochondrial damage[29]]. As previously reported [12] presence of more polyunsaturated fatty acids in the liver is easily prone to the attack by free radicals leading to loss membrane integrity. This condition could cause oxidative damage to the cell membrane which might be theplausible reason for the observed increase of serum enzyme markers leakage from the cytoplasm. Thus the observed results imply the hepato cellular damage occurred in obese animals could be attributed to the sustained ROS formation, LPO and impaired Ca2+ homeostasis and depleted antioxidant defense system and these mechanisms may have significant role in the MSG induced obese mice. The findings are in line with the previous reports [37,35] suggesting that hepatic damage is one of the obesity associated complications. In support of the present biochemical results, the histological examinations also evidenced the hepato cellular injury in MSG obese mice[photos-2a& 2b] indicating necrosis of hepatocytes and nucleus, ,nuclear swelling,pyknotic nuclei,dilated central vein with lysed blood cells and disrupted hepatic architecture in obese mice which confirm with previous studies in MSG injected rats[12,31,35-38] and mice[41]. The observation further agrees with previous findings[30] in msg treated rats.

Heartwood crude extract of P.marsupium administration to the MSG obese mice for 30, 60 and 90 days could significantly reduce the activities of serum enzyme markers (Tables and figure: 3) indicating that the extract was able to condition the hepatocytes by preventing the leakage of intracellular enzymes is clearly understood. The protective function could be attributed to the phyto chemicals present in the heartwood extract. Similarly the hepato protective effect of PMS extract is also investigated in STZ [23] diabetic rats and CCL4 induced heaptotoxicity [39].

As suggested earlier, the anti diabetic and anti oxidant potential of PMS could inactivate and scavenge the free radicals and ROS formation, indicate the anti peroxidative role of the extract. Further, the reduction in the enzyme activity and MDA level was gradual with the increase of duration of extract administration in the obese animals. Additionally, effective control of AST, ALT and ALP activity in the extract fed obese animals (Table 1-3) points towards an early improvement in the secretory mechanism of hepatocytes. Extract administration could prevent cellular damage and would obviously lead to restore the structural and physiological functions of liver(photos 3a&3b) .In support of the present histological observations[37,40] also reveal the limited extent of histological damages in extract fed obese animals is due to the protective effect of heartwood extract. It can be considered as an expression of the functional improvement of the hepatic injury in the extract fed animals. As reported recently, pterostilbene is a potent antioxidant besides polyphenolic compounds [42-44] present in theheartwood, could play crucial role in reducing the oxidative stress associated obesity in male mice, therebyameliorating MSG induced alteration in biochemical parameters and improving hepatocellular damage. Phytochemical studies in PMS further, have shown that flavonoids , and glycosides afford antioxidant potential enhancing antioxidant enzymes activity to scavenge reactive species,maintaining the stability and integrity of plasma membrane[38] and further saponins in the heart wood extract could act against glutamate toxicity [42].

In conclusion, on the basis of overall findings it is clearly implicated that despite the ability of MSG induced obesity associated with hepatic damage, PMS heartwood extract could ameliorate obesity related metabolic dysfunctions in liver through its bio -active phytoconstituents.

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Serum Enzyme Markers

Table-1: SGPT/AST level in U/L.

GROUP	DURATION			
	0-Day	30Day	60Day	90Day
CONTROL	39.90 ±2.044	44.60 ±1.601	58.73 ±0.784	62.40 ±2.252
MSG-OBESE	65.60 ±0.525	75.76 ±1.746	112.40 ±1.785	115.20 ±0.855
OBESE+EXTRACT	65.60 ±0.525	62.20 ±2.780	58.50 ±2.073	56.80 ±1.183

Note: Values are Mean ±SD of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Duncan's test. Comparisons were made between: a) Group I vs Group II, b) Group II vs Group III. P<0.05,s-significant.

Table-2: SGOT/ALT level in U/L.

GROUP	DURATION			
	0-Day	30Day	60Day	90Day
CONTROL	36.60 ±0.935	35.80 ±1.374	38.30 ±0.663	39.55 ±1.050
MSG-OBESE	66.50 ±1.223	69.40 ±0.484	76.60 ±0.914	80.50 ±0.350
OBESE+EXTRACT	66.50 ±1.223	52.40 ±1.629	51.20 ±1.721	44.20 ±0.920

Note: Values are Mean ±SD of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Duncan's test. Comparisons were made between: a) Group I vs Group II, b) Group II vs Group III. P<0.05,s-significant.

Table-3: ALP level in U/L

GROUP	DURATION			
	0-Day	30Day	60Day	90Day
CONTROL	22.50 ±1.775	43.50 ±0.379	47.90 ±5.693	68.80 ±0.824
MSG-OBESE	93.80 ±0.712	114.50 ±1.359	167.20 ±1.471	175.40 ±1.435
OBESE+EXTRACT	93.80 ±0.712	85.60 ±1.692	75.70 ±3.443	53.50 ±4.837

Note: Values are Mean ±SD of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Duncan's test. Comparisons were made between: a) Group I vs Group II, b) Group II vs Group III. P<0.05,s-significant.

Table-4: Malondialdehyde (MDA) level nmol/ mg protein

SAMPLES	LIVER			
Duration (Days) / GROUP	0 Day	30th Day	60th Day	90th Day
I – CONTROL	4.34 ± 0.632	5.58 ± 0.228	6.19 ± 0.497	6.80 ± 0.704
II - MSG-OBESE	9.25 ± 0.387	10.50 ± 1.414	13.20 ± 1.624	16.50 ± 0.894
III – MSG OBESE+ EXTRACT	9.25 ± 0.387	6.50 ± 0.894	5.90 ± 0.800	4.75 ± 1.072

Note: Values are Mean ±SD of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Duncan's test. Comparisons were made between: a) Group I vs Group II, b) Group II vs Group III. P<0.05,s-significant.

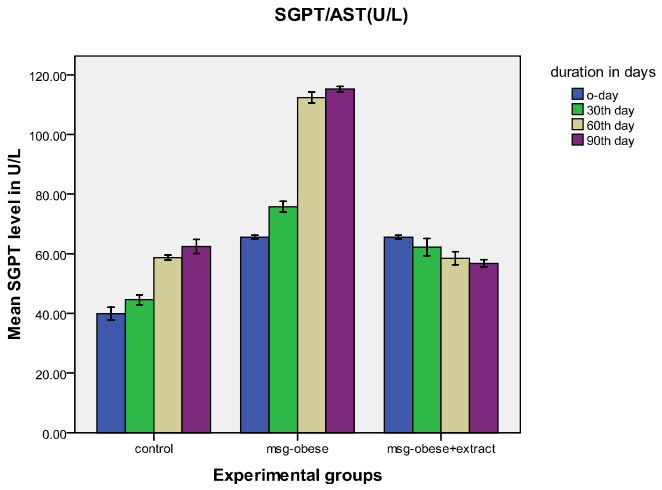


Figure-1

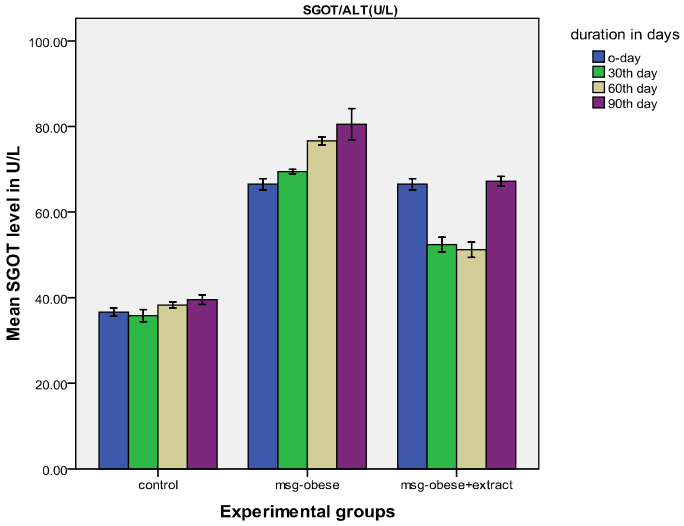


Figure-2

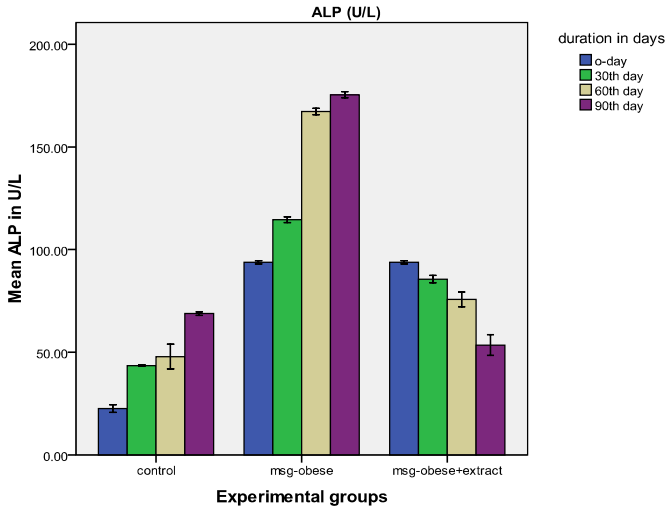


Figure-3

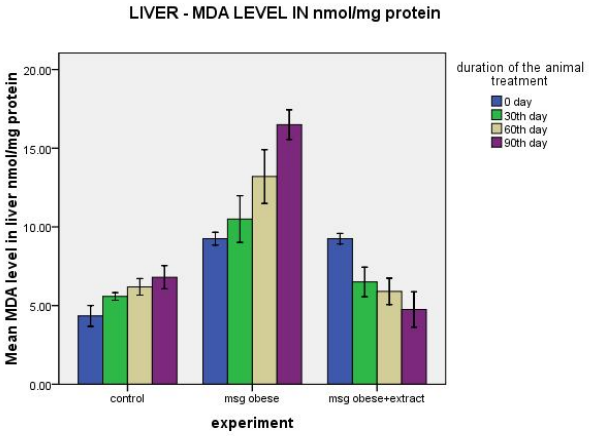
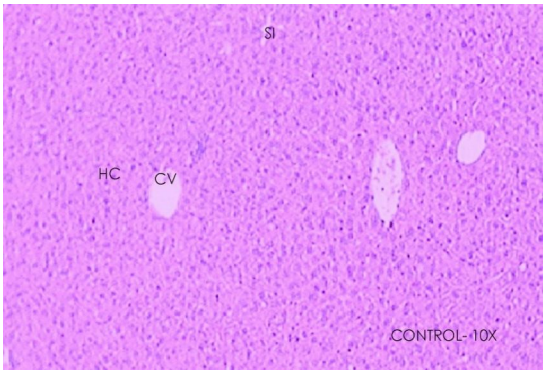
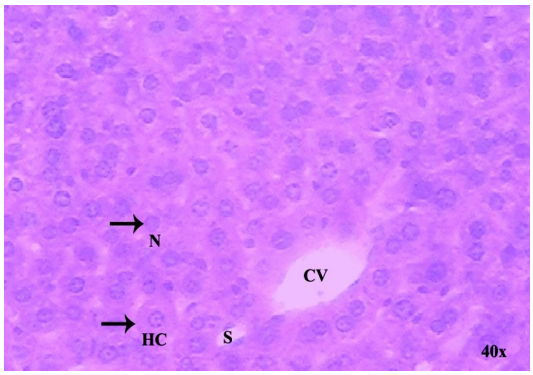


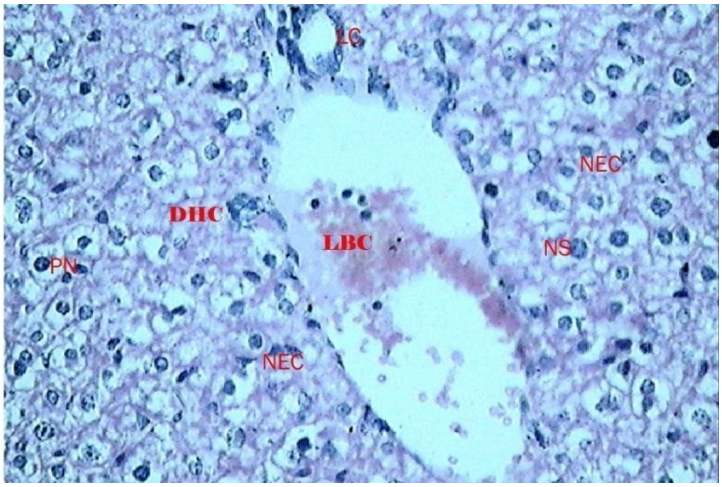
Figure-4





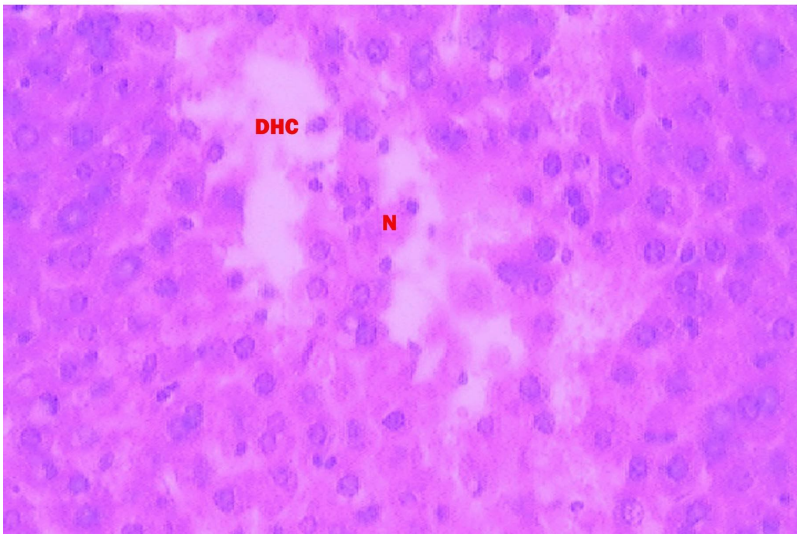
(1a) **(1b)**

Photomicrographs of Control liver sections of albino mice (10X) showing normal histological liver architecture with central vein (CV), hepatocytes(HC), sinusoids(SI)



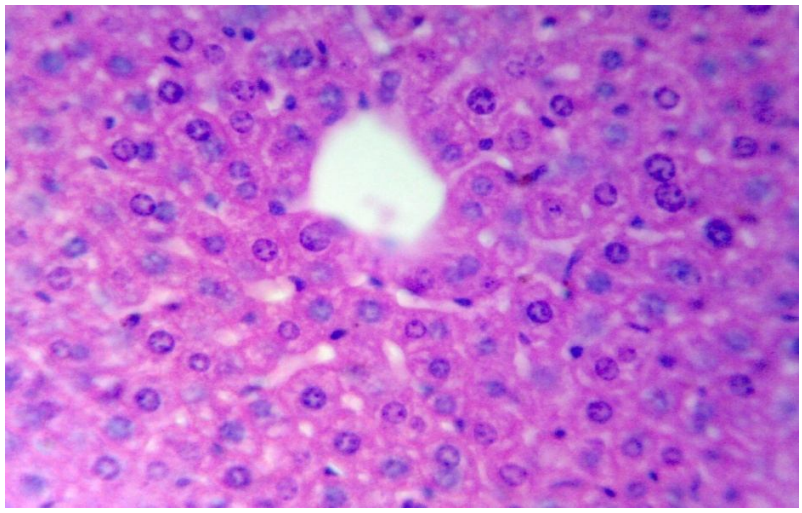
(2a)

Photomicrographs of liver sections of MSG –Obese mice (40X) showing dilated central vein with Lysed Blood Cells(LBC), Necrosis , Degenerated Hepatocytes(DHC), Necrosis(NEC), Pyknotic Nuclei(PN).



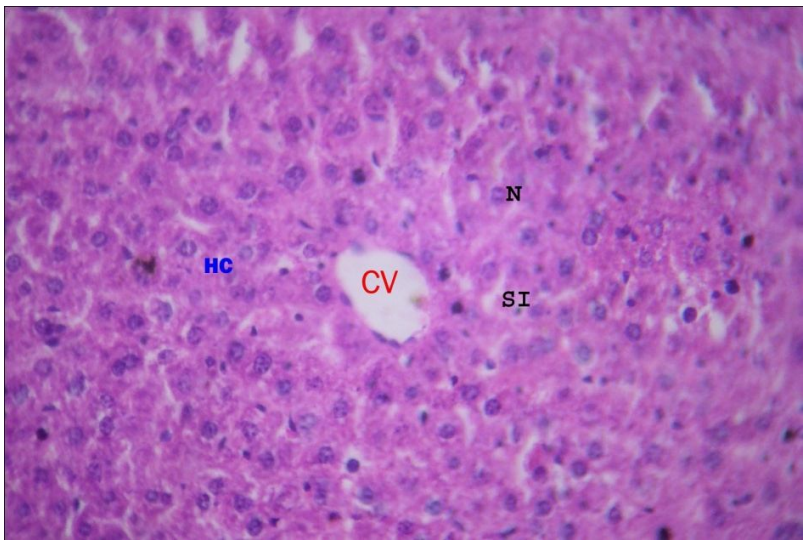
(2b)

(40X) showing loss of hepatocellular architecture with inflammation and atrophic changes



(3a)

Photo micrographs of liver sections from PMS extract fed MSG obese groups



(3b)

3a &3b showing mild distortions in hepatic architecture in the extract fed MSG obese mice

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RESEARCH ARTICLE

Effect of Blood Meal Substitution for Groundnut oilcake in the Fish Feed on Growth and Nutritive Composition of *Catla catla* and *Labeo rohita*

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ABSTRACT

A 60 day feeding trial was conducted to assess the influence of the goat blood meal substitute on the growth and nutritive values of *Catla catla* and *Labeo rohita.* The percentage growth increase of both fishes after 60 days of feeding with groundnut oil cake and rice bran partially substituted with goat blood was measured. The body length and weight of the experimental fishes were found to be significantly increased as a result of feeding with experimental diets. It reflects the same trend in the relative and specific growth rates also.Increased significantly in both major carps as a result of diet supplemented with blood meal. The blood and muscle total free sugar values showed increasing trend in both the experimental fishes. The protein contents of the experimental fishes were also found to be significantly increased (63 and 51% in blood; 74 and 38% in muscles of catla and rohu respectively).In contrast, the blood and muscle cholesterol levels have been decreased in the range of 32 - 62 % by the experimental diet. It suggests the superiority of the experimental feed over the conventional feeds. Therefore the cooked goat blood is considered as an effective, readily available protein supplement for aquaculture.

Key words: *Catla catla, Labeo rohita,* groundnut oil cake, rice bran, blood meal, cholesterol, sugar and protein.

INTRODUCTION

Fish is an important dietary animal protein source in human nutrition [1]. Fish food is only the source to combat the demand of protein rich diets in relation to vegetables and other animal diets. But the availability of fish is very

limited from both marine and fresh water sources. The percaput availability of fish is only 2.08 kg, while the minimum quantity recommended to meet out the basic nutritional requirement from fish is 11 kg/yr/caput [2]. Hence it is very essential to develop fresh water fish culture industries to meet out the self-sufficiency of nutritive foods and to overcome the malnutrition. Indeed aquaculture offers strong potential to meet the national fish demand [3]. Fish species generally require higher levels of dietary proteins for their optimum growth than poultry and cattle [4]. But the use of conventional feeds like groundnut oilcake, rice bran etc., not only provides less amount of proteins than the amount required to the optimal growth but also expensive. The cost of feed ingredients limits the growth and expansion rate of aquaculture industry [5]. Hence, it is important to use alternative feeds with high level of dietary proteins at low cost. A variety of feed ingredients of both plant and animal origins are used in the preparation of artificial diets in intensive aquaculture. Though fish meal continues to be one of the major protein sources in the preparation of fish feed, their rising cost, uncertain availability and fluctuating fish quality has led to the search for alternative protein sources to sustain the fish culture [6]. Soybean meal (SBM) is rich in quality proteins among the plant proteins used in fish feeds [5]. However the use of SBM in aquaculture industries is limited so far due to its demand for human as well as animal consumption [7]. This has also encouraged the search of cheap and alternative protein source for replacing the conventional food stuff without reducing the nutritional quality [8].

The use of leaf meal as a suitable substitute to reduce the cost is also receiving increasing attention by fish nutritionists around the world. Improved growth performance with leucaena as a dietary protein source has been reported for *Java tilapia* [9]. Leucaena, a protein rich trophical leguminous plant growing in Bangladesh, has been widely used in many parts of the trophics as a protein supplement in ruminent and poultry feeds [10]. The partial substitution of ingredients of some plants such as *Hydrilla verticillata, Channa fragilis*, cotton seed, drumstick leaves and gingili oil cake with the groundnut oil cake was also tested and which showed 32% of growth increase in *Oreochromis mossambicus* [11]. The *alfalfa* and *Trifolium sp*., proved to be an acceptable protein source for Chinease blunt snout. The inclusion of alfalfa in the diets resulted in better palatability, increase feed intake and improvement of flesh quality of fish [12]. Water hyacinth has been used in fish culture for many years in many countries and which also proved better performance in fish growth [13]. However, so far none of them has attracted the aquaculture industries that much. Hence in the present study we have taken an effort to formulate a supplementary feed for fresh water fishes (*Catla catla* and *Labeo rohita*) using goat blood as a supplementary feed.

MATERIALS AND METHODS

The young ones of major carps *Catla catla* and *Labeo rohita* were purchased from a fish farm located in a village of Tanjore district, Tamilnadu, South India and brought to the lab in a plastic bag containing the pond water. The fish were acclimatized in stocking tanks filled with river Cauvery water separately. During this period, the fish were fed with groundnut oil cake and rice bran in 1:1 ratio.

Experimental Design - One of the two tanks of 100 liters capacity was marked as control and the other as experimental. After 24 hour starvation each tank was introduced with 10 fingerlings of *Catla catla.* The length and weight of the control fish were ranged from 6.9 to 9.4 cm with a mean of 8.1 $\pm$ 0.8 cm and 4.4 to 12.0 gm with a mean of 8.05 $\pm$ 2.50 gm. So also the length and weight of the young fish in experimental tank were ranged from 7.0 to 9.3 cm with a mean of 7.95 $\pm$ 0.82 cm and 5.5 to 12.0 gm with a mean of 7.92 $\pm$ 2.32 gm. In the same way another set of two tanks were introduced with the fingerlings of *Labeo rohita* in both control and experimental tanks. The total length and weight of the control fish were ranged from 6.9 to 10.0 cm with a mean of 8.11 $\pm$ 0.88 cm and 4.0 to 10.0 gm with a mean of 6.4 $\pm$ 1.79 gm and in the experimental tank the parameters were ranged from 7.2 to 8.8 cm with a mean of 7.84 $\pm$ 0.51 cm and 4.0 to 9.0 gm with a mean of 6.39 $\pm$ 2.03 gm respectively. All the tanks were connected with an aerator and covered with nylon nets.

Feed preparation - The experimental diet was prepared by mixing groundnut oil cake, rice bran and goat blood at the ratio of 2:2:1 (400:400:200 gms) in the appropriate volume of distilled water until obtaining a semisolid nature after well cooking. 10 gm of *Agar agar* powder was also added to the diet to serve as the binding material. While still hot the cooked diet was fed through an extruder having perforated disc with 2 mm diameter holes. The noodles were sundried and broken to pellets of 5 -20 mm length. The control diet was also prepared by the same way as that of the experimental feed by cooking groundnut oil cake and rice bran at the ratio of 1:1(500:500 gms).

Feeding protocol - The fish of control tanks were fed with pellets of control diet (Groundnut oil cake and rice bran in 1:1 ratio) whereas the experimental fish of both species were fed with the pelleted diet partially supplemented with goat blood. All the fish were fed twice daily and the left over were removed two hours after providing food. The water quality parameters like temperature, dissolved oxygen, salinity and pH were also monitored to be 28 ± 1°C, 3.35 ± 0.1 ml/lit., 1.34 ± 0.1% and 7.9 ± 0.01 respectively. The experiment was conducted for the period of 60 days.

Growth studies - The total length and weight measurements of fish were recorded 0^{th}, 30^{th} and 60^{th} days of the experimental period and the growth rates were calculated using the following formulae.

$$\text{Relative growth rate (RGR \%)} = \frac{\text{Growth (Final wt. - Initial weight)}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR (\%/day)} = \frac{\text{Growth (Final wt. - Initial weight)}}{\text{Duration (days)}} \times 100$$

Biochemical analysis - At the termination of the experiment, the fish fed with both control and experimental diets were sacrificed after collecting sufficient amount of blood by severing caudal region. The flesh of each fish was collected in the appropriate container according to the investigation to be carried out. The total free sugars, cholesterol and protein were estimated adopting the procedures of Roe [14]. Zarrow et al., [15] and Gornal et al., [16] respectively.

Statistical analysis - The length and weight measurements and the biochemical parameters obtained at initial and after the experimental periods were ananysed by students' t' test for mean difference between control and experiment as described by Parker [17].

RESULTS

Length and Weight - Data pertaining to total length and weight of Catla and Rohu at initial (0 day) and after feeding for 30 and 60 days are given in the table 1. The initial mean total length of catla was 8.1 ± 0.8 cm. After feeding with control diet for 30 and 60 days, the mean total length increased to 8.67 ± 0.94 and 9.44 ± 1.05 cm respectively. On the other hand the young catla fish fed with experimental diet, showed a significant increase in both the periods. The mean total length increased from 7.95 ± 0.82 to 9.11 ± 0.93 and 10.69 ± 1.11 cm. In fact, the percentage increase was observed to be greater in the experimental fishs when compared with the control fish group. The mean total length increase of Rohu fed with experimental diet also showed the same trend as that of experimental catla where a significant increase were found in both periods (from 7.84 ± 0.51 to 9.4 ± 0.56 and 11.38 ± 0.58 cm after 30 and 60 days of feeding). Similarly the weight of catla and rohu were also increased at both periods after feeding with control and

experimental diets. The weight measurements of experimental fishes were found to be more than the control fishes as shown in the table 1.

Growth rates - Fig.1 revealed the relative and specific growth rates of Catla and Rohu after feeding with the experimental diet for 60 days. The percentage increases of RGR and SGR were found to be 32 and 31% respectively in catla whereas the same were increased to 115 and 123% in rohu.

Organic constituents of Blood and Muscles - After 60 days of feeding with control and experimental diets, the percentage increase/decrease of total free sugars, total proteins and cholesterol levels of blood and muscles of both the fishes were shown in the table 2 and fig.2. The % increase of total free sugars in the blood of catla and rohu were observed to be 11 and 04% respectively which were not significant. At the same time the increase of total free sugars were found to be 75 and 74% in the muscles which were significant values. The same increasing trend was noted for the total protein levels of the both fishes in both blood and muscles (63 and 51% in blood of catla and rohu; 74 and 38% in muscles of the both respectively). Here the % increase of total proteins in blood and muscle of both fishes were significant. In contrast the cholesterol levels of experimental Catla and Rohu have been decreased significantly in both blood and muscle samples (32 and 36% in blood and 62 and 58% muscles). Here also the percentage decreases were observed to be significant.

DISCUSSION

In the present investigation an experimental diet was prepared by partially substituting the groundnut oilcake with goat blood (containing larger amount of protein) and 1% *Agar agar*. The control group of catla and rohu were fed with the conventional diet of oil cake and rice bran at 1:1 ratio for 60 days whereas the experimental groups of both fishes were fed with the above said experimental diet for the same period. The diets were accepted by both groups and utilized for growth in that the percentage relative and specific growth rates increased significantly. Infact, the experimental diet enhanced the growth of the fishes significantly. A 32 and 31% increase of growth rates (RGR and SGR) were observed in experimental catla whereas the same was found to be 115 and 123% in the experimental rohu (Fig.1). It is also notable that the growth rates of rohu was found to be greater than that of catla for the diet substituted with goat blood. The growth enhancement by blood meal substitute is lining with some early investigations [11,18]. The percentage increase of total protein content in the experimental fishes is also the strong evidence for the growth increment due to the blood meal substitute. The percentage increase in protein level of blood and muscle were observed to be 63 and 74 % in *Catla catla* and 51 and 38% in *Labeo rohita.* The significant increase in the protein content of the muscles of the experimental fishes shows their higher nutritive values to compare the control groups. It is also notable that, the catla fed with experimental diet has lesser growth rates than the rohu fed by the same diet which shows that the rohu has faster growth by blood meal substitute. On the other hand the percentage increase of protein accumulation in the muscles of catla is 74 % whereas it seems to be only 38 % in rohu for the experimental feed. This is also in agreement with earlier reports of Nandeesha et al., [19] in *Catla catla* and Das et al., [20] in *Labeo rohita.* The present study also reveals the increasing trend in total free sugars of blood and muscles in the fishes fed with the experimental diet (Fig.2). It is the evidence for that the blood meal being a good energy source. In contrast the cholesterol level of both experimental groups has been decreased significantly and which suggests the superiority of goat blood meal over the conventional diet of groundnut oil cake and rice bran (Fig.2). Therefore the cooked goat blood is considered as an effective, readily available protein supplement for aquaculture. Further study in this direction may provide new insight in the commercial scale preparation of potential fish feed.

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Table 1: Mean values of total length and weight of *Catla catla* and *Labeo rohita* at initial and after 30 and 60 days of feeding with control and experimental diets.

Parameter	Animal	Control (days)			Experiment (days)		
		0	30	60	0	30	60
Length (cm)	*Catla catla*	8.10±0.80	8.67±0.94	9.44±1.05	7.95±0.82	9.11±0.93	10.69±1.11
	Labeo rohita	8.11±0.88	9.33±0.83	11.01±0.91	7.84±0.51	9.40±0.56	11.38±0.58
Weight (gm)	*Catla catla*	8.05±2.50	8.92±2.72	9.96±2.91	7.92±2.32	9.10±2.57	10.42.±2.84
	Labeo rohita	6.40±1.79	6.80±1.84	7.35±1.87	6.39±2.03	7.42±2.38	8.79±2.80

Table 2: Mean levels of Biochemical parameters of Blood are Muscles of *Catla catla* and *Labeorohita* after 60 days of feeding with Control and experimental diets.

SAMPLE	PARAMETERS	ANIMAL			
		Catla catla		*Labeo rohita*	
		Control	Experiment	Control	Experiment
BLOOD (mg /100 ml)	Total free sugar	203.27±40.65	226.27±63.62	323.45±39.76	337.84±44.22
	Total protein	16.4±2.95	26.8±4.13	23.0±4.02	34.8±3.43
	Cholesterol	104.01±22.94	70.98±22.37	299.28±48.49	192.85±38.39
MUSCLES (mg/gm wet. wt)	Total free sugar	39.94±8.57	69.93±17.23	18.38±5.38	31.96±9.96
	Total protein	337.6±24.09	588.47±40.93	395.2±30.22	545.6±44.27
	Cholesterol	62.8±8.2	23.66±7.38	97.8±11.68	40.66±11.19

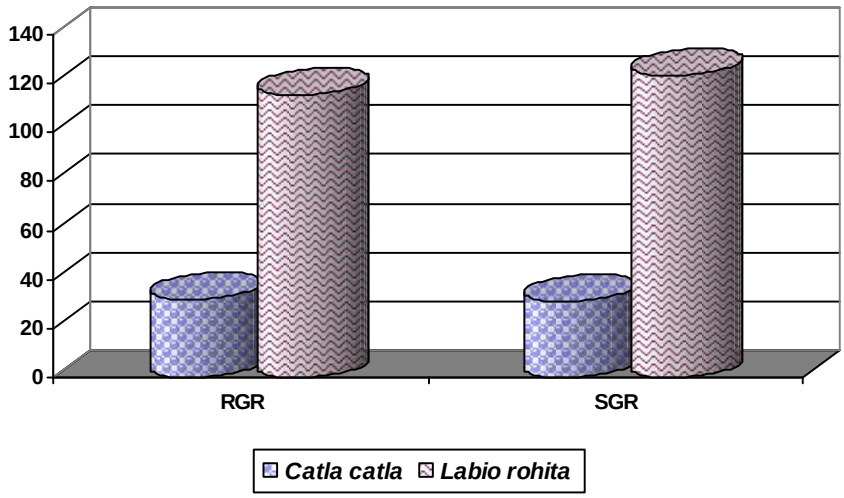

Fig 1: Percentage increase of relative and specific growth rates in the fishes (*Catla catla* and *Labeo rohita)* after 60 days of feeding with experimental diets

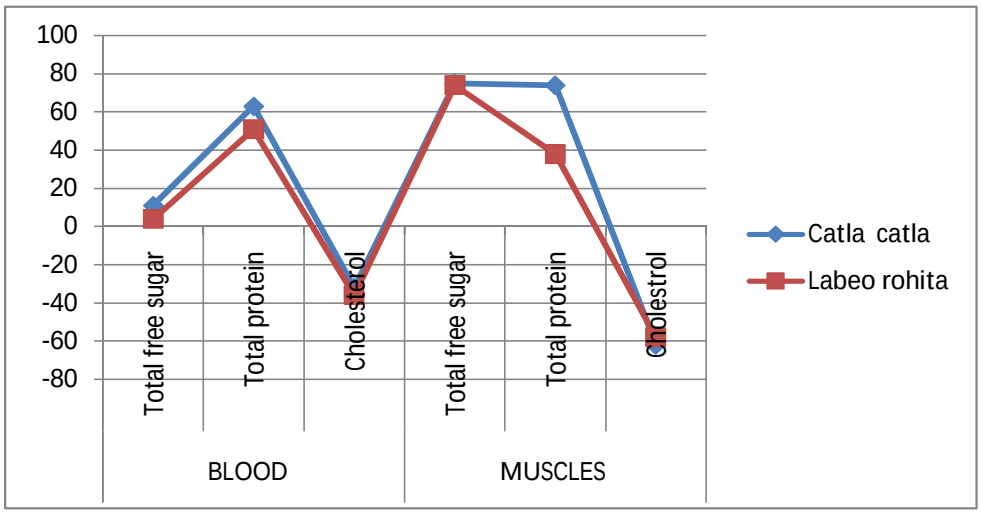

Fig 2: Percentage increase/decrease of biochemical parameters (total free sugar, total protein and cholesterol) of blood and muscles in the fishes of *Catla catla* and *Labeo rohita* after 60 days of feeding with experimental diets.

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RESEARCH ARTICLE

Heavy Metal Contamination in Bhadravathi Town, Shimoga District, Karnataka, India.

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ABSTRACT

Heavy metals have been used by humans for thousands of years. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years. Cadmium compounds are currently mainly used in re-chargeable nickel–cadmium batteries. Cadmium emissions have increased dramatically during the 20th century, one reason being that cadmium-containing products are rarely re-cycled, but often dumped together with household waste. Cigarette smoking is a major source of cadmium exposure. In non-smokers, food is the most important source of cadmium exposure. Recent data indicate that adverse health effects of cadmium exposure may occur at lower exposure levels than previously anticipated, primarily in the form of kidney damage but possibly also bone effects and fractures.

Key words: Cadmium, Mercury, Arsenic and human health

INTRODUCTION

We know very little about the effect that pollution has on the oceans, but we continue to dispose off chemicals, sewage and garbage into it at an unprecedented rate. Most people likely do not even know what types of pollutants reach the oceans. There may be billions of people unconcerned about ocean pollution and hence this problem.

Truly, the fish catch from the sea will tend to bio concentrate the pollutants to finally reach the humans. Toxic pollutants in the Sea ecosystem have massive impacts on the plants and animals. Heavy metal poisoning (such aslead and mercury) from industrial effluents accumulate in the tissues aquatic life.Many individuals in Europe already exceed these exposure levels and the margin is very narrow for large groups. Therefore, measures should be taken to reduce cadmium exposure in the general population in order to minimize the risk of adverse health effects. The general population is primarily exposed to mercury *via* food, fish being a major source of methyl mercury exposure, and dental amalgam. The general population does not face a significant health risk from methyl mercury, although certain groups with high fish consumption may attain blood levels associated with a low risk of neurological damage to adults. Since there is a risk to the fetus in particular, pregnant women should avoid a high intake of certain fish, such as shark, swordfish and tuna; fish (such as pike, walleye and bass) taken from polluted fresh waters should especially be avoided. There has been a debate on the safety of dental amalgams and claims have been made that mercury from amalgam may cause a variety of diseases. However, there are no studies so far that have been able to show any associations between amalgam fillings and ill health.

The general population is exposed to lead from air and food in roughly equal proportions. During the last century, lead emissions to ambient air have caused considerable pollution, mainly due to lead emissions from petrol. Children are particularly susceptible to lead exposure due to high gastrointestinal uptake and the permeable blood–brain barrier. Blood levels in children should be reduced below the levels so far considered acceptable, recent data indicating that there may be neurotoxic effects of lead at lower levels of exposure than previously anticipated. Although lead in petrol has dramatically decreased over the last decades, thereby reducing environmental exposure, phasing out any remaining uses of lead additives in motor fuels should be encouraged. The use of lead-based paints should be abandoned, and lead should not be used in food containers. In particular, the public should be aware of glazed food containers, which may leach lead into food. Exposure to arsenic is mainly *via* intake of food and drinking water, food being the most important source in most populations. Long-term exposure to arsenic in drinking-water is mainly related to increased risks of skin cancer, but also some other cancers, as well as other skin lesions such as hyperkeratosis and pigmentation changes. Occupational exposure to arsenic, primarily by inhalation, is causally associated with lung cancer. Clear exposure–response relationships and high risks have been observed.

MATERIALS AND METHODS

Bhadravathi is an industrial town and taluk in the Shivamogga District of Karnataka state, India. The town is spread over an area of 67.0536 square kilometres (25.8895 sq mi) and has a population of 180,392 as per the census held in 2010. Bhadravati had a population of 160,392. Males constitute 51% of the population and females 49%. Bhadravati has an average literacy rate of 74%, Bhadravathi lies in the central part of the Karnataka State, in the south-east corner of the Shivamogga district. The latitude and longitude coordinates of Bhadravathi town are 13°50′24″N 75°42′07″E13.840°N 75.702°E Coordinates: 13°50′24″N 75°42′07″E13.840°N 75.702°E.Bhadravathi is at an altitude of 597 metres (1,959 ft) above sea level.

The survey was conducted by selecting Four sites in Bhadrvathi Taluk i.e. Kadadakatte, Agardhalli, Kudli and Holehonnur. The sites were selected on the basis of pollution, rainfall pattern and soil characteristics of the regions. For the purpose laboratory analysis and the details of samples were tabulated. The survey was conducted in the Months of January 2009 – December 2009. During the study period primary information about the Sampling sites and water samples are collected for determination of heavy metals in the samples.

RESULTS

The parameters were furnished in the table-1. The results indicate that the water considerably varies from location to location. The water is mainly characterized by a relatively P^H of around 5.5 to 7.1 for the samples collected. Cadmium was detected it shows excessive limit. There is a major cause for aquatic organisms and also on human health, especially its an polluted water. High in acidity due to Industrialization and human activities. There was low values of EC were found in the locations. The BOD levels are high in the study sites, i. e in Site 3. According to the study the high amount of cadmium found in the Site 3, followed by Site 4, Site 1 and Site 2, the Mercury levels was high in the Site 3, moreover Arsenic also showed the high degree of standards in the study Site. From this investigation the Site 3 shows high degree of pollution.

DISCUSSION

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain. Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted.Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium(Cr), thallium (Tl), and lead (Pb).

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Table :1.Physico -Chemical parameters and Heavy metal analysis of Sampling Stations in and around Bhadravathi

Sampling Sites	PH	TDS	EC	BOD (Mg/l)	Alkalinity	Cd (mg/l)	Mercury (mg/l)	Arsenic (mg/l)
Site-1	6.2	779	1150	127	233	3.5	0.012	0.4
Site-2	6.0	950	1410	156	100	3.0	0.21	0.8
Site-3	5.2	690	1030	878	140	7.3	1.1	1.4
Site-4	7.8	1200	1820	578	380	6.8	0.9	0.7

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RESEARCH ARTICLE

Evaluation of Protective Potential of *pterocarpus marsupium* Roxb. in the Tissues of MSG-Induced Obese Male Albino Mice -*Mus musculus.*

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ABSTRACT

The aim of the study was to evaluate the potential of the heart wood of *Pterocarpus marsupium*(PMS) on oxidative stress markers in the tissue of MSG - induced obese male mice. Obesity was induced by neonatal injection of Monosodium glutamate (MSG),the widely used food additive and the study was performed in male obese mice to assess the oxidative stress indicators such as lipid peroxidation and protein oxidation in liver, brain and serum tissues of obese mice. The experimental design consisted of three groups, control(I), MSG obese(II), MSG obese with extract administered(III) for three months. The results showed significant($P<0.05$) elevated level of malondi aldehydes(MDA) and protein carbonyl content(PCC) measured as index of, lipid peroxidation and protein oxidation, which was sustained throughout study period(0 to 90days) in the MSG-induced obese animals as compared to the control. In the extract fed MSG obese groups , there had been significant reduction in MDA and PCC levels revealing the potential of inhibiting free radical formation, scavenging the peroxyl radicals so as to reduce the oxidative stress caused by the toxicity of glutamate in MSG obese. The heart wood extract effect was in a time-dependent manner exhibited by the obese animals. Thus ,the study reveals the tissue protective potential of heartwood extract of PMS which could be attributed to the phytochemicals present in the extract and it might beneficial in preventing later complications in MSG- induced obese animals.

Keywords: Obesity, Monosodium glutamate, Malondi aldehydes, Protein carbonyl, Lipid peroxidation, Protein oxidation, Pterocarpus marsupium, Heart wood , Peroxyl radicals.

INTRODUCTION

The prevalence of obesity has significantly increased during the last decades reaching epidemic proportions in many countries. Like other developed countries, in India also there has been more incidence of obesity among children and adults and one of the reasons reported from many investigations is that the higher consumption of the flavor enhancer , Monosodium glutamate (Ajinomotto). It is the sodium salt of glutamic acid, a food additive used in a wide range of processed foods in the industry, as well as in cooking also to improve the palatability of meals. Previous report[1] revealed the link between MSG and obesity due to lesion in the hypothalamus region. Also data from animal and human studies indicate that MSG intake could lead to obesity associated with hyperglycemia, hyperinsulinemia, impaired glucose tolerance, dyslipidemia, fatty liver, cardiovascular diseases and pathological conditions[2-5]. Several studies showed toxic effects of MSG in various regions of the CNS[6-8], liver[9,10]and kidney[11], mainly by generation of reactive oxygen species (ROS) and resulting oxidative stress. Chronic administration of MSG (4 mg/g body weight and above) was found to induce oxidative stress in experimental animals[8-12].

Based on the literature cited, in the current work MSG was used to induce obesity in the male albino mice, *Mus musculus* and the study was designed to elucidate oxidative stress indicators, measuring lipid peroxidation (LPO) and protein oxidation (PO) in liver, brain and serum of MSG obese mice and to evaluate the protective potential of heart wood (crude) extract of *Pterocarpus marsupium* (PMS) in obese animals.

Medicinal plants have been used as dietary supplements for body weight management and control in many countries. *Pterocarpus marsupium* Roxb. (Leguminosae) is a large deciduous tree commonly found in hilly regions of India, used in traditional Ayurvedic medicine for the treatment of diabetes mellitus. The phytochemical screening of ethanol extracts of *P. marsupium* wood and bark revealed the presence of alkaloids coumarins, flavonoids, glycosides, terpenoids, tannins, phenols, saponins and steroids[13].There are many studies revealing the potential of anti diabetic, hypolipidimic [14] antioxidant[15], antiperoxidative [16] anti obesity properties of bioactive compounds present in wood and bark[17-19].

MATERIALS AND METHODS

Animals: Forty healthy adult male and female Swiss-strain albino mice (*Mus musculus*) weighing between 20 and 25g were obtained from Kings Institute, Chennai. The mice were housed in animal house with exposure to 10–12 hr of daylight at a relative humidity of 30–70%. They were fed standard mouse feed and were given water *ad libitum*. After the animals bred, the new born male pups were used for the study. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee.

Chemicals: Monosodium glutamate **(MSG)** was purchased from the commercial shop which was labeled "Ajino-moto". All other chemical reagents were of analytical grades and purchased from Hi-Media (Mumbai).

Plant material: The heartwood of *Pterocarpus marsupium* (PMS) was collected from Idukki district in Kerala. PMS heartwood was washed with distilled water, shade dried, powdered and stored in an air- tight container.

Preparation of extract: The heartwood of PMS (10g) was cut in to small slices, powdered and it was soaked in distilled water (100ml) overnight, boiled and filtered. The dosage used was 0.25 ml of 10% aqueous extract as fixed by Farzana[20].

Induction of Obesity in Male Albino mice: Subcutaneous injection of MSG (4mg/gm.b.wt) was given to the five days old neonatal pups on alternative days, each received six doses of MSG. The male mice were tested for diabetes from fourth week after the last dose of MSG injection by measuring blood glucose level. Animals of each group were weighed weekly. Development of obesity was confirmed by body mass index calculation at third month of the study period.

Experimental design

Exposure : The mice were divided into four groups each with 10 to 15 pups.
Group I : Served as control (male pups) which had 0.5% saline
Group II : MSG obese control-Male pups injected subcutaneously with MSG (4 mg/gm.b.wt.)
Group III: MSG obese male mice + crude extract of *P.marsupium* heartwood administered orally (0.25 ml / gm.b.wt /day) with a baby oral feeding tube.

Sample Collection: At the end of 3rd month after the last MSG dose, the animals were fasted overnight, noticing the blood glucose levels, animals were sacrificed by cervical dislocation. The blood was taken by heart puncture and centrifuged at 3000 rpm for 10 min to obtain the serum and brain, liver, and kidney were removed, washed in ice-cold physiological saline (0.9%), blotted and weighed. 100 mg of each tissue was homogenized in 0.1M Tris- HCL buffer (pH 7.4) at 4ºC in a homogenizer. The homogenate was centrifuged at 3000 rpm for 30 min at 4ºC in a refrigerated centrifuge to obtain the supernatant and it was used for the estimation of biochemical parameters such as malondialdehyde (MDA) and protein carbonyl content (PCC) which are measured as an indicator of the extent of lipid peroxidation(LPO) and protein oxidation(PO) mechanism respectively. Protein estimation was carried out by Lowry *et al* [21].

Biochemical analysis

Oxidative stress Biomarkers:

a. Lipid peroxidation**:** Lipid peroxides expressed as Malondialdehyde (MDA) was estimated using thiobarbituric acid reagent (TBARS) as described by Ohkawa[22] .
b. Protein oxidation: protein carbonyl level was measured by the method of Levin[23].

Statistical analysis: Data are expressed as mean ± S.D. Statistical comparison was done by one-way analysis variance(Anova). P<0.05 was considered as statistically significant.

RESULTS

Lipid peroxidation: The data in table-1 revealed that there was more than two fold increase (113.13%) in liver at 0 day in MSG –obese mice as compared to its related control and this increase of MDA was 78.3% at 90th day in MSG-obese animals.Meanwhile, the PMS extract fed obese animals showed significant (P<0.05) reduction in MDA level as compared to MSG –obese mice. The reduction was initially by 29.7%, 36.2% and finally 48.6 % at the end (90th day). Similarly, data in table-1 revealed a significant increase of MDA level in brain of obese mice where is the mean values being 2.45±0.14, 4.56±0.19, 5.25±0.03 and 6.87±0.81 at 0, 30 ,60 and 90 days respectively when compared to control and threefold increase of MDA level (180.4%) at 90th day in MSG-obese mice. There was slight increase in MDA in the control animals from 0 to 90 days (0.85±0.07, 1.67±0.17, 1.79±0.06, 1.96±0.14 respectively) indicating the age related effect. As the data shown, there had been considerable decreased MDA level in the PMS extract fed obese animals compared to the MSG-obese group. Initially the decrease was 12.2% (30day) and at 90 day it was by 55.9% reduced in the extract fed group.Serum MDA concentration is summarized (Table-1) for control, MSG-obese animals and PMS extract fed obese animals. There was a significant (P<0.05) increase of serum MDA in obese group as compared to the

control where it was twofold increase initially as the mean values showed 2.91±0.06, in MSG- obese and it was 0.99±0.13 nmol/mg protein in 0 day control. At 90th day the MSG obese animals exhibited 39.2% increase of serum MDA. Meanwhile, with the advancement of time slight increase of serum MDA in the control animals itself was noticed.However, serum MDA level of MSG-obese animals which were administered PMS extract showed 28.5% (30day), 47% (60day) and 48% (90day) reduction of MDA. The significant decrease of serum MDA level was finally 62.5% in the extract fed mice as compared to 90 day MSG-obese animals.The crude extract of PMS heart wood had significantly ($p<0.05$) reduced the MDA levels in all the tissues of extract fed MSG obese animals and it revealed that the reduction was gradual and in a time-dependent manner. It can be observed that the extract brought down MDA level by 48.6% 55.9% ,62.5% decrease of MDA in liver, brain and serum respectively at the end of study period (90th day) in the extract fed MSG-Obese mice when compared to the MSG-Obese control. The 90th day MDA level in liver was closer to the 0day level in normal control mice .Similarly in brain also the same effect was observed which was closer to the 30thday MDA level of normal control mice and also in serum, the decreased effect on 60th day was closer to its relative normal control.

Protein oxidation

Results in table-2 illustrate the concentrations of protein carbonyl (PCC) in liver, brain and serum of the experimental animals.Compared to control, liver protein carbonyl level was significantly elevated in MSG-obese mice from 0 day to 90 days (36.9%) with the advancement of time. Age related effect on protein oxidation was also noted in the control animals (0 to 90 days old). MSG-obese mice exhibited pronounced increase in carbonyl content (4.20±0.28, 4.85±0.08, 5.25±0.89, 5.75±0.44 nmol/mg protein) at 0, 30, 60 and 90 days respectively. Administration of PMS extract in the MSG-obese mice significantly reduced protein oxidation by 50% at 90 days of treatment as compared to the MSG-obese group.Data presented in Table-2 revealed the significant increase of carbonyl content in the brain tissues of MSG-obese animals from 0 day to 90 days as compared to control. Meanwhile, in the control group age related effect on protein oxidation was also noted from 0 to 90 days old animals. MSG-obese group exhibited significantly increased carbonyl content (2.1±0.126, 2.75±0.447, 3.02±0.021 and 4.20±0.282 nmol/mg protein respectively) and the increase was more pronounced at 90th day by 50%.

It can be noticed that the PMS extract fed obese mice showed marked decrease of protein carbonyl levels by 24.8%, 42.8% and 54.8% respectively compared to obese animals . The reduction was significant ($P<0.05$) and time dependent effect of the extract was noticed.Table-2 represents the serum protein carbonyl concentration in the control, MSG-obese and PMS extract fed obese animals from 0 to 90 days. As compared to control, MSG-obese mice exhibited significantly higher levels of protein oxidation which was shown by the mean values 1.01± 0.06, 1.66± 0.04, 2.02 ±0.01 and 3.90 ±0.34nmol/mg protein at 0, 30, 60 and 90 days respectively. Further, there was slight increase of carbonyl content in the control animals also (0 to 90 days).

PMS extract fed obese group had shown significant ($P<0.05$) reduction of carbonyl content in serum compared to the MSG-obese mice. Due to the extract effect the reduction was 20.8%, 28.7% and finally 35.6% at 30, 60 and 90 days respectively in the extract fed MSG-obese animals. Protein oxidation level in the extract fed obese showed significant ($P<0.05$) reduction by 50%, 54.8%, 35.6% in liver, brain and serum tissues respectively after 90 days of extract treatment.

DISCUSSION

Obesity is a major contributor to several metabolic disturbances related to oxidative balance. Oxidative stress is a biochemical disequilibrium propitiated by excessive production of free radicals (FR) and ROS, which provoke

oxidative damage to biomolecules. Fat accumulation correlated with systemic oxidative stress in humans and mice [24]. The significant increase of MDA level , an indicator of free radical generation and end product of LPO along with the increase in protein oxidation(PO) which is measured as protein carbonyl content level (PCC) was noted in the tissues of MSG obese mice (table-1,2) indicating the sustained and prolonged MSG induced oxidative stress in animals. It can be noticed that the remarkable increase was with the advancement of time which further reveals the enhanced free radicals and reactive oxygen species formed under obese status. Similar to the results obtained, there are many reports suggesting that oxidative stress leading to oxidative damage to the tissues and organs due to MSG toxicity.

Studies of[10-12,25] reported that MSG-induced obesity resulted in metabolic disorder associated with oxidative stress and tissue pathology due to enhanced lipid peroxidation. It is well documented that oxidative stress is associated with obesity and LPO and PO are reported to be involved in the development of obesity[24] which is revealed in the current study. Oxidative injury due to toxic effects of MSG in rodents is by the generation of reactive oxygen species(ROS)causing mitochondrial membrane damage is reported[10-12]. It is suggested that considerable amounts of polyunsaturated fatty acids that are prone to damage by free radicals through oxidative stress in the tissues which involved in high metabolic activity[27,28]. In line with this report, data obtained imply the fact that oxidative damage in the tissues of obese animals could be also due to more accumulation of fat and enhanced hyperglycemia as reported earlier[29].

Increased ROS production from accumulated fat also leads to increased oxidative stress in blood could severely affect other organs also including the liver, skeletal muscle, and aorta[24]. The ROS formed in obese animals initiated peroxidation of polyunsaturated acyl chains of membrane phospholipids result in the formation of lipid – derived aldehydes is also evidenced[30]. It has been reported previously that the administration of MSG caused increased lipid peroxidation in hepatic tissue due to increase in blood glutamate and glutamine levels[31] thereby causing hyper-lipidemia and hyperglycemia, which are important factors for the onset of oxidative stress[32,33]. It is also evidenced in the plasma and erythrocytes in obese diabetic patients[34]. Increased oxidative stress in blood can affect other organs such as endothelium, liver and in obese subjects[33,35] claiming that the reason could be due to the increased level of glutamine which led change in redox potential of the cell and dysregulation of membrane integrity in MSG administered mice. Additionally [36]suggest that the activation of glutamine gated cation channels may be another source of OS and enhanced LPO leading to neuronal damage in MSG administered rats. Further it is showed that since brain is more vulnerable to OS injury due to its high rate of oxidative metabolic activity and high content of polyunsaturated fatty acids and easily susceptible for LPO, it led neuronal damage[27,28]. In confirmation to the present findings neuronal damage reported by[37,6] and studies of[8]also indicate that MSG ingestion in mice caused significant increase of LPO due to increased glutamate level through the activation N-methyl-D aspartate (NMDA) receptors causing more influx of ca^{2+} ions into the cells damage the mitochondrial function resulting into aberrant formation of free radicals / ROS. Based on the afore mentioned investigations, the MSG obese animals exhibiting prolonged LPO caused enhanced oxidation of cellular proteins resulted in more protein carbonyl formation. Studies of [38] imply that due to the end products formed in LPO the protein damage and inactivation of membrane bound enzymes indicate the cellular damage in obese animals. It is suggested that proteins have many different and unique biological functions, oxidative modifications to proteins can lead to diverse functional consequences increased oxidative stress in accumulated fat is an important pathogenic mechanism of obesity-associated metabolic syndrome. The plasma lipid peroxidation products and protein carbonyl groups were found significantly higher in obese diabetics[39]. Also studies indicate that oxidative modification of proteins increase with the development of obesity is an intracellular indicator of tissue damage which is in accordance with the current result.

Administration of crude extract of PMS heartwood in MSG induced obese mice resulted in significant decrease of MDA and PCC levels (table-1, 2) revealing considerable protective effect of the extract taken place with the increase of treatment time (30-90 days). The results indicate the inhibition of peroxidation and oxidation of the biomolecules lipids and proteins which may be important for the alleviation of the resulting oxidative damage occurred in the tissues of obese animals. It could be attributed to the phyto constituents flavonoids and polyphenols effect against free radical mediated oxidative mechanism. Studies indicate that free radical generation is inhibited by[40] and anti oxidant activity of polyphenols could improve the status of oxidative stress biomarkers [41] and it exhibit anti-obese property[19]. Further findings [42]suggests that phenolic compounds in plants can act as metal chelator and directly scavenge active oxygen species[43]. In addition to the findings of hepato protective activity[43,44] it has been also investigated plant flavonoids could protect neuronal cells from oxidative stress preventing ca^{2+} influx and directly lowering ROS level by increasing intracellular glutathione level (GSH). As reported recently, pterostilbene is a potent antioxidant[44] and also other flavonoids and polyphenolic constituents present in the heartwood exhibited in the current work it is clearly indicated that extract administration exhibited its potential of anti peroxidative mechanism in the tissues of obese animals and the amelioration was found to be increased with time i.e. time- dependent effect. Another reason can be attributed to the correlate with the anti diabetic effect of PMS[15] that might cause the reduction of auto-oxidation of glucose and increased glucose uptake, enhancing lipolysis[14] which are also the sources involved in obese animals. A number of studies have revealed various health benefits of plant polyphenols having some potential efficacy for preventing obesity [42]. In accordance with the reports mentioned, data obtained reveal the protective potential of the heartwood extract treatment in MSG obese mice was implicated through the potent bio- active principles present in the extract.

Thus the present observations in the study can be concluded that the protective role of PMS heartwood extract is due to its anti peroxidative , anti-oxidative and antidiabetic properties of the phyto-chemicals exerted its potential to reduce the oxidative load in the obese animals to prevent against oxidative injury to the tissues in MSG obese animals. Hence the ameliorative effect of the extract could be beneficial to reduce or prevent the possible later complications of obesity in MSG obese animals.

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Table: I - Malon Di Aldehyde (MDA) Level in the Experimental Animals (nmol/ mg protein)

SAMPLES	LIVER				BRAIN				SERUM			
Duration (Days) / **GROUP**	**0 Day**	**30th Day**	**60th Day**	**90th Day**	**0 Day**	**30th Day**	**60th Day**	**90th Day**	**0 Day**	**30th Day**	**60th Day**	**90th Day**
I - CONTROL	4.34 ± 0.632	5.58 ± 0.228	6.19 ± 0.497	6.80 ± 0.704	0.85 ± 0.079	1.67 ± 0.173	1.79 ± 0.067	1.96 ± 0.147	0.99 ± .131	1.17 ± 0.068	1.75 ± 0.064	1.93 ± 0.048
II - MSG-OBESE	9.25 ± 0.387	10.50 ± 1.414	13.20 ± 1.624	16.50 ± 0.894	2.45 ± 0.142	4.56 ± 0.1945	5.25 ± 0.031	6.87 ± 0.816	2.91 ± 0.061	3.84 ± 0.038	3.96 ± 0.151	4.05 ± 0.442
III – MSG OBESE+ EXTRACT	9.25 ± 0.387	6.50 ± 0.894	5.90 ± 0.800	4.75 ± 1.072	2.45 ± 0.142	2.15 ± 0.126	2.33 ± 0.143	1.08 ± 0.097	2.91 ± 0.061	2.08 ± 0.294	1.54 ± 0.252	1.52 ± 0.300

Note: Values are Mean ±SD of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Duncan's test. Comparisons were made between: a) Group I vs Group II, b) Group II vs Group III. P<0.05, S-significant.

Table: II- Protein Carbonyl Content (Pcc) in the Experimental Animals (nmol/mg protein)

SAMPLES	LIVER				BRAIN				SERUM			
Duration (Days) / **GROUP**	**0 Day**	**30th Day**	**60th Day**	**90th Day**	**0 Day**	**30th Day**	**60th Day**	**90th Day**	**0 Day**	**30th Day**	**60th Day**	**90th Day**
I - CONTROL	2.70 ± 0.034	3.54 ± 0.023	3.80 ± 0.050	4.23 ± 0.077	0.06 ± 0.017	0.33 ± 0.020	0.28 ± 0.025	0.40 ± 0.017	0.12 ± 0.017	0.21 ± 0.062	0.34 ± 0.045	0.52 ± 0.030
II - MSG-OBESE	4.20 ± 0.282	4.85 ± 0.089	5.25 ± 0.894	5.75 ± 0.447	2.10 ± 0.126	2.75 ± 0.447	3.02 ± 0.021	4.20 ± 0.282	1.02 ± 0.068	1.66 ± 0.040	2.02 ± 0.017	3.90 ± 0.340
III – MSG OBESE + EXTRACT	4.20 ± 0.282	3.02 ± 0.021	2.75 ± 0.447	2.10 ± 0.126	2.10 ± 0.126	1.58 ± 0.376	1.20 ± 0.357	0.95 ± 0.202	1.01 ± 0.066	0.80 ± 0.069	0.72 ± 0.051	0.65 ± 0.051

Note: Values are Mean ±SD of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Duncan's test. Comparisons were made between: a) Group I vs Group II, b) Group II vs Group III. P<0.05, S-significant.

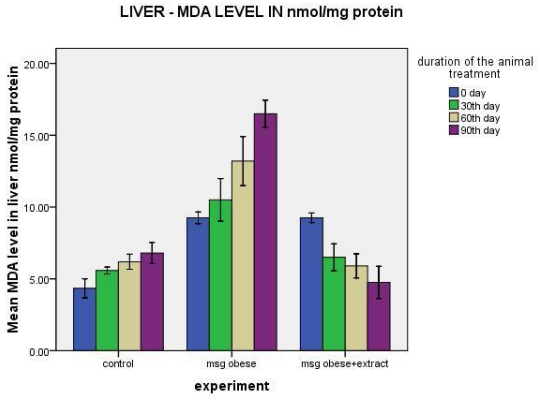
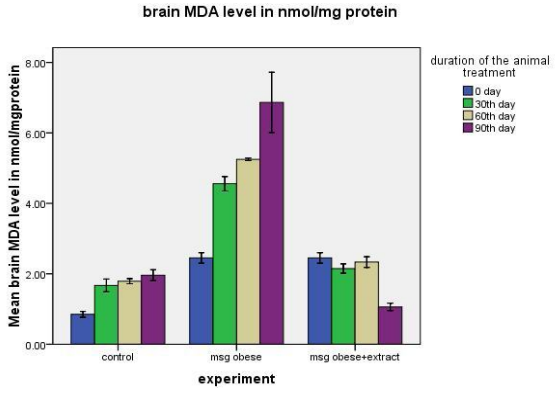


Figure :1.1



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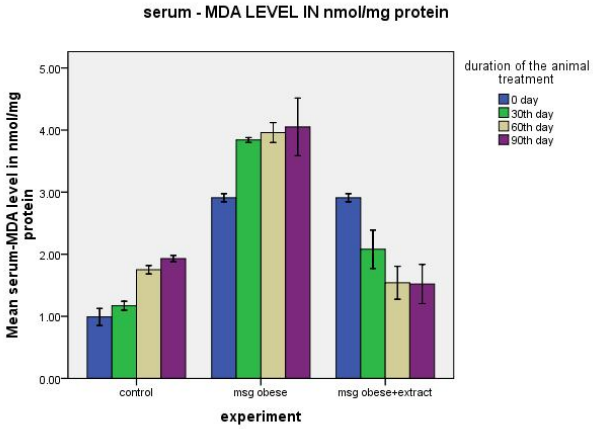

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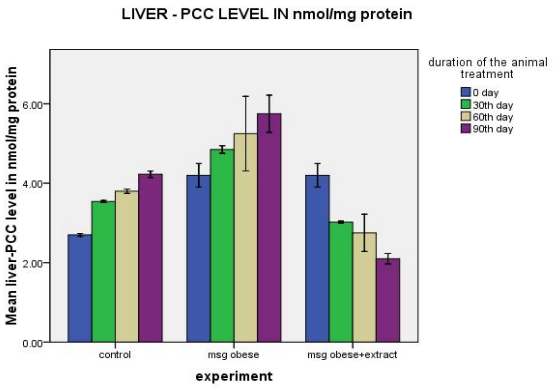

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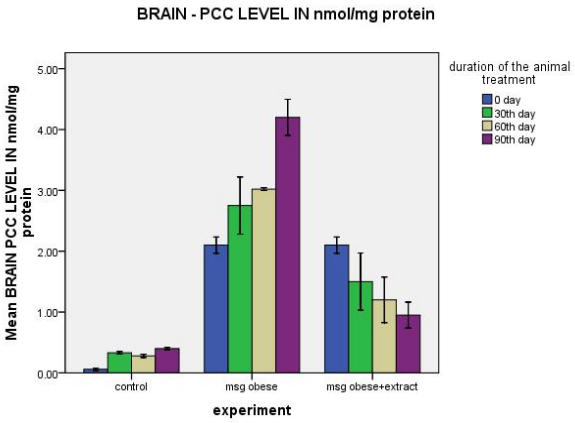

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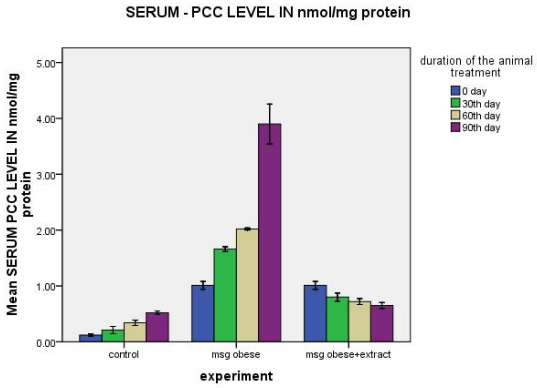

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RESEARCH ARTICLE

Heavy Metals Contamination in Soils and Food Crops in the Vicinity of Orathupalayam Reservoir in Tiruppur, TamilNadu, India.

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ABSTRACT

This study was designed to investigate heavy metal contamination levels of soils and food crops grown in the vicinity of the Orathupalayam reservoir in Tiruppur. Comparison of heavy metal content in agricultural soils in industrial effluents irrigated area with control site reveals substantial accumulation of metals in the former one. The accumulation of metals in food crops in industrial effluents irrigated area was observed in the order of Fe > Mn > Zn > Cu > Cr > Ni > Pb. Concentration of Pb and Ni were higher than the safe limits of Indian Prevention of Food Adulteration standards in all the examined food crops.

Key words: Heavy metals, food crops, human health, Orathupalayam reservoir

INTRODUCTION

The utilization of industrial effluents for irrigation activities in agricultural land is on the rise particularly in peri-urban areas of developing countries like India. In general, the concentration of heavy metals in industrial effluents are high and long-term use of these waste waters on agricultural lands often results in the massive build-up of the elevated levels of these metals in soils (Rattan et al. 2001). However, extent of accumulation of metals in wastewater-irrigated soils depends on the period of its application (Bansal et al. 1992). Certain plants can accumulate heavy metals in their tissues and uptake increases generally in plants that are grown in areas with increased soil contamination with heavy metals (LeCoultre, 2001). Although some heavy metals such as Cu, Zn, Mn, and Fe are essential in plant nutrition, many heavy metals do not play any significant role in the plants physiology. Plants growing in a polluted environment can accumulate the toxic metals at high concentration causing serious risk to human health when consumed (Vousta et al. 1996; Alloway 1990). Consumption of food crops enriched with toxic

heavy metals may cause serious health hazards through food-chain magnification (Rai and Tripathi 2008). Since, food chain contamination is one of the major routes for entry of metals into the human and animal population (Rattan et al. 2005), monitoring the bioavailable pools of metals in contaminated soils has generated a lot of interest.

The River Noyyal is one of the most intensively used river basin sources of the pollution in the river basin are effluent from domestic and urban settlements, various chemicals from the industries and land based activities. In general, the pollution is load is high in the river Noyyal due to the poor runoff and lack of adequate rainfall in the catchment area. In a day, the River Noyyal receives around 75000m^3 to 100000 m^3 of untreated/partially treated industrial effluents from the dyeing and bleaching industries which are located on the banks of the river. Besides, nearly 126 lakh liters of untreated sewage also discharged into the River Noyyal from Tiruppur city alone (Senthilnathan 2004). In this background, the present study is focused to assess the status of heavy metal distribution in surface sediments of River Noyyal in Tiruppur region.

MATERIALS AND METHODS

Three main food crops (*Sorghum vulgare, Zeamays (Maize), Sataria italica*) samples were collected from adjacent agricultural sites in the vicinity of Orathupalayam reservoir during 2009 in the month of August. The collected plant sample was washed with running tap water to remove extraneous matter and then with distilled water. After washing the plant material was blotted dry, finally chopped and air dried. After this the individuals were rinsed with distilled water and were then chopped / cut into roots, stems and leaves using plant cutter. The samples were then kept in oven at 70°C till constant weight. The oven dried samples were grinded for homogenization and passed through a sieve of 2 mm mesh size. The sieved samples were stored in pre-cleaned plastic containers. Some of the metals such as Fe, Mn, Cu, Cr, Zn, Pb and Ni were estimated using Atomic Absorption Spectrometer (AAS).

Exactly 1g of dried homogenized plant sample was weighed using a monopan digital balance and transferred to 500 ml round bottom flask. To this a little amount of distilled water was added to make slurry was swirled gently. Then to this a mixture of each 10 ml nitric acid, 2 ml Perchloric acid and 1 ml Sulphuric acid was added respectively (Allen et al. 1986). The sample was then digested for 1 hour at 90°C. Appearance of brown fumes during digestion was followed by the addition of 2.5 ml of 30% Hydrogen Peroxide. Further processing was carried out in the same manner as soil samples. The samples were then transferred to pre-treated, laboratory cleaned, plastic vials and stored at 4°C till further analysis. The digested samples were aspirated in the Atomic Absorption Spectrophotometer (AAS, Perkin Elmer) and absorbance was noted from which estimations were made for each metal concentration in mg/kg. For quality assurance, replicate samples, blanks and standardized reference materials were used during analysis.

RESULTS AND DISCUSSION

Surface water analysis revealed severe contamination in River Noyyal by heavy metals. Besides the dyeing and bleaching industries which are located on the both sides of River Noyyal, huge level of domestic waste water and solid wastes also discharged into the River this is consistent with metal content in surface water and food crops grown in farms irrigated from this river. Farms which irrigated by this water were contaminated due to regular discharge of massive quantity of effluents and domestic waste water. Additionally, partial or no treatment is applied to the industrial discharges to detoxify the wastewater draining into the river. Even though, common effluent treatment plants (CETPs) are committed to treat these effluents in the environmentally safe manner but certainly it has been affected by the lack of functional efficiency.

Significant difference between raw effluents and surface water samples might be due to the reason that the effluents gets diluted in the water during the flow of the water in the river. Comparison of heavy metal content in agricultural soils in dyeing and bleaching industrial effluents irrigated area with control site reveals substantial accumulation of metals in the former one. It suggests prolonged use of effluents are suspected source of substantial accumulation of heavy metals in irrigated soil. In effluents, among the heavy metals studied Fe was in recorded maximum level (2450 µg/l) and Mn recorded in minimum concentration (220 µg/l). Based on the concentration of metals content in effluents was observed in the order of Fe > Zn > Cr > Ni > Cu > Pb > Mn. However, in effluents irrigated soil metal distribution was found in the order of Fe > Mn > Zn > Cu > Cr > Ni > Pb (Table).

Consumption of food crops contaminated with heavy metals is one of the important pathways for intake of toxic substances into the human body of which some become apparent only after several years of exposure (Bahemuka and Mubofu 1999; Ikeda et al. 2000). The consumption of heavy metal contaminated food can deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, intrauterine growth retardation, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Iyengar and Nair 2000; Turkdogan et al. 2003). Accumulation of heavy metals in three investigated plants (*Sorghum vulgare, Zeamays, Sataria italica*) were observed in the order Fe > Mn > Zn > Cu > Cr > Ni > Pb (Table 2). Irrespective of different parts of plants studied, Fe level was observed in the range from 62 to 2015 mg/kg. Among the plant parts, root showed maximum accumulation of Fe followed by seed, stem and leaf. However, In *S. Vulgare* Fe level in leaf is slightly higher than the stem. Except *S. vulgare,* the Fe accumulation was observed in the order of root > seed > stem > leaf. In root, maximum level of Fe (2015 mg/kg) content was observed in *S. vulgare* followed by *Zeamays* (188 mg/kg) and *S. italica* (178 mg/kg).

Mn concentration was found maximum (155 mg/kg) in root (*Zeamays)* followed by root of *S. italica* (139 mg/kg). As like Fe, Mn is mainly concentrated in root followed by seed, stem and leaf, respectively. In seeds, maximum level of Mn was observed in *Zeamays* (105 mg/kg) followed by *S. vulgare* (96 mg/kg) and *S. italica* (58 mg/kg). Mn content in studied food crops observed in the order of: *Zeamays* > *S. vulgare* > *S. italica.* Irrespective of different parts of plants studied, Mn level was observed in the range from 22 to 155 mg/kg. Mn was found to accumulate in leafs and seeds in both control and effluents irrigated crops possibly due to their requirement in metabolism (Vishnu et al. 2008). Cu level in studied plant parts varied from 6.5 to 58 mg/kg and its accumulation observed in the order of: *S. italica* > *S. vulgare* > *Zeamays.* In roots, maximum level of Cu was observed in *S. italica* (58 mg/kg) followed by *Zeamays* (41 mg/kg) and *S. vulgare* (35 mg/kg). Except *S. italica,* Cu accretion was slightly higher in stem than leaf in *Zeamays* and *S. italica*. Among the plant parts studied, maximum level of Cu was recorded in root whereas minimum level was recorded in stem. Cu level in all three food crops found to be low in editable portions and higher level in root indicates possible movement from effluents contaminated soils. Xiong and Wang (2005) found that Cu concentration in the shoots was significantly influenced by Cu concentration in soil and increased markedly with an increase in the soil Cu concentration.

Concentration of Cr in different parts of plants was found in the range between 1.9 and 26 mg/kg. In stem, maximum level of Cr (12.8 mg/kg) was observed in *S. italica* followed by *Zeamays* (3.6 mg/kg) and *S. italica* (3.1 mg/kg). Among the plant parts investigated leaf recorded low level of Cr. In general, Cr level was observed in the order of: *S. italica* > *S. vulgare* > *Zeamays.* In root, maximum level of Cr (26 mg/kg) content was observed in *S. italica* followed by *S. vulgare* (21 mg/kg) and *Zeamays* (18 mg/kg). Maximum and minimum level of Pb was recorded in root and stem as 5.4 (*S. vulgare)* and 0.4 mg/kg (*Zeamays)*, respectively. Irrespective of plants studied, Pb was found in the range from 0.4 to 5.4 mg/kg. In leafs, maximum level of Pb (1.9 mg/kg) was observed in *Zeamays* followed by *S. vulgare* (1.1 mg/kg) and *S. italica* (0.9 mg/kg). Pb level was observed in the order of: *Zeamays* > *S. vulgare* > *S. italica.* In roots,

maximum level of Pb (5.4 mg/kg) was observed in *S. vulgare* followed by *Zeamays* (4.9 mg/kg) and *S. italica* (3.1 mg/kg). Pb accumulation in the studied food crops exceeded several times higher than the threshold level permissible for human. Higher level accumulation of Pb in edible portion of studied food crops may cause health risks to exposed population through food chain transport. Higher level Ni content in seeds of S. *vulgare* and *Zeamays* (Maize) than *S. italica* implies preferential accumulation of this element in selected crops. Earlier studies also found that the greatest potential health risk to consumers of wastewater-irrigated vegetables was from Pb, Cd and Ni while no risk was observed due to intake of Zn, Cr and Cu (Al Jassir et al. 2005; Muchuweti et al. 2006; Singh et al. 2010).

Zn accumulation in investigated plant parts figured from 17 to 78 mg/kg and observed in the order of: *S. italica* > *Zeamays* >*S. vulgare.* In roots, maximum level of Cu was observed in *S. italica* (58 mg/kg) followed by *Zeamays* (41 mg/kg) and *S. vulgare* (35 mg/kg). Except *S. italica,* Cu accretion was slightly higher in stem than leaf in *Zeamays* and *S. italica*. Among the plant parts studied, maximum level of Zn was recorded in root and minimum level was found in stem. Many fold high concentration of Zn in edible portion suggests possible health risk to humanity. Higher level of Zn intake may lead to tachycardia, vascular shock, dyspeptic nausea, vomiting, diarrhea, damage of hepatic parenchyma and interference in the Cu metabolism (Barone et al. 1998; Salgueiro et al. 2000). Reed et al. (1991) found Zinc in the leaf increased with increasing sludge application. Ni level was observed maximum (5.4 mg/kg) in root (*Zeamays)* followed by root of *S. vulgare* (4.8 mg/kg). Ni content in investigated plants was found in the order of: *Zeamays* > *S. vulgare* > *S. italica.* Except *S. italica,* the Ni accumulation was observed in the order of root > seed > stem > leaf, whereas Ni level in leaf of *S. italica* (1.1 mg/kg) is moderately higher than the stem (0.6 mg/kg). In roots, maximum level of Ni (4.8 mg/kg) was observed in *Zeamays* followed by *S. vulgare* (4.8 mg/kg) and *S. italica* (2.9 mg/kg).

The accumulation of metals in food crops in industrial effluents irrigated area was observed in the order of Fe > Mn > Zn > Cu > Cr > Ni > Pb. The comparison of the mean levels of these metals with the permissible limits of Indian Prevention of Food Adulteration Act 1954 (Awashthi 2000) shows that the concentration of Pb and Ni were above the safe limit in all the examined food crops. However, analysis of variance not yielded any significant difference in heavy metal concentration between studied crops but significant difference was observed between various parts of plants. Earlier studies also found treated or partially treated wastewater irrigation posing threat of metal accumulation in plants (Dudka and Miller, 1999; Fytianos et al. 2001; Gothberg et al. 2002). They also showed that the metal concentration vary in the different parts of the plants. Both in the control as well as effluents irrigated food crops, preferential accretion of metals in roots and seeds of studied food crops suggest gradual translocation of these metals from roots to seeds. In contrast, earlier study conducted by Akdeniz et al. (2006) also demonstrated that the sewage biosolid applications may not cause any significant increase in heavy metal levels in leaf and seed of *S. vulgare*.

CONCLUSION

Surface water analysis revealed severe contamination of surface water in River Noyyal by heavy metals. Apart from dyeing and bleaching industries which are located on the both sides of River Noyyal, huge level of domestic waste water and solid wastes also discharged into the river this is consistent with metal content in surface water and food crops grown in farms irrigated from this river. Farms which irrigated by this water were contaminated due to regular discharge of massive quantity of effluents and domestic waste water. Comparison of heavy metal content in agricultural soils in dyeing and bleaching industrial effluents irrigated area with control site reveals substantial accumulation of metals in the former one. It suggests prolonged use of effluents might cause substantial accumulation of Cu, Cr, Zn and Pb in irrigated soil. The accumulation of metals in food crops in industrial effluents irrigated area was observed in the order of Fe > Mn > Zn > Cu > Cr > Ni > Pb. The comparison of the mean levels of

these metals with the permissible limits of Indian Prevention of Food Adulteration Act 1954 shows that the concentration of Pb and Ni were above the safe limit in all the examined food crops.

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Table 1. Descriptive statistics for selected metals concentration in effluent (µg/l), soil samples in effluents irrigated and control sites (mg/kg).

Metals	Raw effluents (µg/l)	Surface water (µg/l)	Guidelines for irrigation water (µg/l) (Ayers and Westcott, 1985)	Effluents irrigated Soil (mg/kg)	Control Soil (mg/kg)	Guidelines for soil (mg/kg) (Ewers 1991; Pendias and Pendias, 1992)
Fe	2450	1060	500	684	594	50000
Mn	220	114	200	421	395	2000
Cu	450	220	17	72	38	100
Cr	820	515	550	31.8	21	100
Zn	1125	661	200	84	52	300
Pb	260	140	65	8.4	4.1	100
Ni	620	270	1400	16	11	50

Table 2. Accumulation of heavy metals in different parts of three agricultural crops (*Sorghum vulgare, Zeamays, Sataria italica*) in effluents irrigated region (all values in mg/kg)

Heavy metals	*Sorghum vulgare*				*Zeamays (Maize)*				*Sataria italica*				Indian safe limit[a]	FAO/ WHO limit[b]
	Root	Stem	Leaf	Seed	Root	Stem	Leaf	Seed	Root	Stem	Leaf	Seed		
Fe	215	115	128	164	188	94	62	142	178	84	68	112	-	-
Mn	112	52	37	96	155	67	32	105	139	36	22	58	-	-
Cu	35	6.5	7.8	18	41	8.7	11.6	22.8	58	13.2	10.8	19.4	30	73.3
Cr	21	3.1	2.7	13.4	18	3.6	1.9	9.6	26	12.8	5.9	16.2	20	2.3
Zn	78	24	20	42	63	17	24	38	55	17	22	26	50	9.4
Pb	5.4	0.8	1.1	1.8	4.9	0.4	1.9	2.1	3.1	0.8	0.9	1.4	2.5	0.3
Ni	4.8	1.1	0.8	2.6	5.4	2.6	1.4	3.1	2.9	0.6	1.1	1.9	1.5	66.9

[a] Awashthi (2000)

[b] FAO/WHO (2001), Joint Codex Alimentarius Commission

Table 3. Accumulation of heavy metals in different parts of three agricultural crops (*Sorghum vulgare, Zeamays, Sataria italica*) in control area (all values in mg/kg)

Heavy metals	*Sorghum vulgare*				*Zeamays (Maize)*				*Sataria italica*			
	Root	Stem	Leaf	Seed	Root	Stem	Leaf	Seed	Root	Stem	Leaf	Seed
Fe	174	102	92	106	162	64	51	112	128	68	58	91
Mn	79	34	17	54	102	51	19	91	84	21	14	36
Cu	24	3.8	5.1	11	28	6.1	8.4	12.4	29	7.5	6.4	11.5
Cr	16	1.2	1.6	8.1	9.2	1.7	1.1	2.8	19	6.1	2.5	8.9
Zn	53	14	9	22	31	12	14	19	42	9	12	14
Pb	0.9	0.2	0.4	0.7	1.1	0.2	0.6	0.5	1.2	0.6	0.5	0.2
Ni	1.4	0.4	0.3	1.4	2.1	1.4	0.7	1.9	1.2	0.2	0.5	0.8

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RESEARCH ARTICLE

Microbial Decolourization and Bioremediation of Molasses Waste Water

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ABSTRACT

Molasses waste water in industries carrying out alcohol fermentation; bakers yeast fermentation etc is high in biochemical and chemical oxygen demand and suspended solids. Presently increasing attention has directed towards utilizing microbial activity for the decolorization and mineralization of molasses spent wash. Owing to this, in the present study, bacterial and yeast species were isolated from molasses and used to treat molasses waste water. Totally five species of bacteria such as *Pseudomonas putida, Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Micrococcus* sp., and four species of yeast *viz*., *Saccharomyces cerevisiae*, *Torulopsis glabrata*, *Candida glabra* and *C. albicans* were isolated from the molasses. For treating the molasses waste water, *P. putida* and *S. cerevisiae* were selected based on the screening process. After incubation the significant decrease was observed in physicochemical parameters values. The BOD and COD levels were reduced nearly 50 to 65% in treated molasses when compared to control. The colour removal was significant in treated effluent. These findings indicate that both *P. putida* and *S. cerevisiae* could effectively be used for the treatment of molasses waste water.

Keywords: Molasses, *Saccharomyces cerevisiae, Pseudomonas putida,* Physicochemical parameters, BOD, COD, melonoidin, effluent decolorization

INTRODUCTION

Molasses is a by-product of the sugar cane, sugar beet and citrus industries. Molasses is a syrup containing mixture of uncrystallizable sugar and non-sugar components [1]. Molasses is one of the major components of growth media used in many industrial processes. Molasses is used as an energy source in a wide range of fermentation processes to grow yeasts, molds and bacteria, which produces brewing, distilling, etc [2]. Molasses waste water is high in biological and chemical oxygen demand (BOD, COD) and suspended solids. Its high COD, total nitrogen and total phosphorous indicates that its disposal into natural water bodies may result in their eutrophication. More than 30 billion liter of molasses spent wash is generated annually by 254 cane molasses based distillery in India alone [3]. Melanoidin is a compound, which is present in molasses spent wash and is highly toxic to microorganisms due to its antioxidant property [4]. Melanoidins are the products of the Maillard reaction between sugar and amino acid produced upon heating [5]. Due to recalcitrant property of melanoidin, its treatment becomes very necessary. It also reduces the fertility of soil by causing manganese deficiency [6]. Melanoidins also reduce the penetration of sunlight though rivers or lakes which as a result decreases both photosynthetic activity and dissolved oxygen concentration. This adversely affects many aquatic plants and animals. Its disposal into soil is equally detrimental, causing inhibition of seed germination and depletion of vegetation by acidified soil [7].

Control of pollution is one of the prime concerns of society today. With economic constraints on pollution control processes, affordable and effective methods have become a necessity. The development of effective and practical methods for removing melanoidin from waste water is expected throughout the world. Although some bacteria [2, 8], fungi [4,6] and yeasts [9] have been reported for the bioremediation of spent wash, there is increasing demands for effective and economical technologies for bioremediation of spent wash, mainly for colour removal. Owing to this in the present work the use of indigenous bacterial and yeast strain for bioremediation of molasses waste water as well as effective removal of colour was carried out.

MATERIALS AND METHODS

The samples were collected from sugar industry at Aranthangi, Tamilnadu, South India. Samples were collected in large sterilized bottles and brought to the laboratory. The molasses samples were filtered through cotton to remove suspended solids. Physico-chemical characteristics were done on the same day when the sample was brought to the laboratory.

Isolation of bacteria and yeasts

10ml of the molasses sample was taken in a 250ml conical flask containing 90ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and serially diluted with sterile distilled water to make different dilutions viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. One ml of 10^{-2} - 10^{-5} dilution was plated in Petri dishes with nutrient agar medium and Sabouraud's dextrose agar medium containing 100ppm of chloramphenicol.

The inoculated nutrient agar plates were incubated at 37^{o}C for 24h or two days and bacteria appearing over the medium were identified based on colony characteristics, Gram staining and by various biochemical tests as given by Bergey's Manual of Determinative Bacteriology [10]. The inoculated Sabouraud's dextrose agar plates were incubated at room temperature for 5-7 days. The yeast colonies developed were identified by staining and microscopic observation.

Physiochemical analysis

The molasses spent wash was filtered through cotton before use and the initial physiochemical analysis was made following the standard methods [11]. The following treatments were employed.

- Molasses spent wash uninoculated – control for physiochemical analysis.
- Molasses spent wash inoculated with *Pseudomonas putida*
- Molasses spent wash inoculated with *Saccharomyces cerevisiae*

Uniform suspensions of the above microorganisms were inoculated separately to the flask with 20% molasses spent wash. The experiment was conducted in duplicates, under controlled conditions (temperature 27 ± 2^0C with light intensities of 1500 lux provided from over head cool white fluorescent tubes) for 20 days. One flask was kept as control without inoculation. After 20 days the cultures were filtered through ordinary filter paper. The filtrate (inoculated and control) were used for physicochemical and decolourization studies.

Estimation of decolourization activity

Melanoidin degradation activity was assayed by measurement of the decrease in optical density as absorbance at 475 nm after diluting with 0.1 M acetate buffer (pH 5.0). The decolourization yield was expressed as the degree of the decrease in absorbance at 475 nm against the initial absorbance at the same wavelength. Decolourization activity was calculated as follows:

$$D\% = 100 \times (A_{fin} - A_{ini}) / A_{ini}$$

Where,

D% = Decolourization percentage

A_{ini} = Initial absorbance

A_{fin} = absorbance after incubation

RESULTS

Bacteria and Yeast flora in the molasses

Bacteria were identified through number of various biochemical tests (Table 1). Totally five species of bacteria such as *Pseudomonas putida*, *Escherichia Coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus* sp. were identified from the effluent sample. Totally four species of yeast such as *Saccharomyces cerevisae*, *Torulopsis glabrata*, *Candida glabra* and *C. albicans* were isolated from the molasses.

Physicochemical characteristics of molasses spent wash

After inoculation and incubation with microorganisms the physicochemical parameters showed significant reduction as that of control, except dissolved oxygen which recorded significant increase. The results of physicochemical analysis are tabulated in Table 2. pH of the molasses spent wash was increased to 6.7 from 4.3, while the free carbon dioxide was reduced by 45.2 % and 64.5% as that of control, dissolved oxygen was slightly increased from 1.34 to 1.45 (8.2 %) and 1.5 (11.2%), after treating with *S. cerevisiae* and *P. putida* respectively.

With respect to the *S. cerevisiae* treatment the following results were observed: there was a 37.8 % reduction of nitrate while, the nitrite was reduced to 29 mg/l from 48 mg/l (39.6 %). The ammonia was reduced to 28 mg/l from 42 mg/l showing 33.3 % of reduction as that of control sample. Total phosphate also showed significant reduction from 90 mg/l to 54 mg/l (40.0%). The degradation of inorganic phosphate (43.6%) was noticed to be more with contrast to

organic phosphate (34.3 %). The minerals Ca and Mg also showed the reduction in their concentration (42.2% and 43.2% respectively). BOD was reduced to 114 mg/l from 240 mg/l (52.5 %) and COD was reduced to 180 mg/l from 320 mg/l (41.9 %).

On treatment with *P. putida* the following results were observed: there was a 47.8 % reduction of nitrate while, the nitrite was reduced to 23 mg/l from 48 mg/l (52.1 %). The ammonia was reduced to 25 mg/l from 42 mg/l showing 40.5 % of reduction as that of control sample. Total phosphate also showed significant reduction from 90 mg/l to 49 mg/l (45.6%). The degradation of inorganic phosphate (47.3%) was noticed to be more with contrast to organic phosphate (42.9%). The minerals Ca and Mg also showed the reduction in their concentration (56.3% and 51.4% respectively). BOD was reduced to 95 mg/l from 240 mg/l (60.4 %) and COD was reduced to 150 mg/l from 320 mg/l (51.6 %).

Decolorization activity

Decolorization activity (Fig. 1) was found to be significant in the molasses spent wash inoculated with both *P. putida* (38%) and *S. cerevisiae* (33%).

DISCUSSION

The present work is essentially focused on the biological process for the treatment of molasses spent wash. Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bioremediation [12]. In the present study *Pseudomonas putida* isolated from molasses was employed for bioremediation which showed significant activity. It is confirmed and supported by earlier findings [8, 13]. They suggested that the polluted habitats harbour mostly *Pseudomonas* because it is having ability to degrade various pollutants from water samples. Bacteria generally are easier to culture and they grow more quickly than fungi. They are more amenable to molecular genetic manipulations. They are able to metabolize chlorinated and other organic contaminants such as oil and mineralize chemicals using them as carbon or energy source [12]. *Saccharomyces cerevisiae* isolated from molasses was also found to be effective in bioremediation of molasses spent waste, but to a lesser extent in comparison to *P. putida*. The bioremediation of molasses waste water by *Saccharomyces* has been reported [9].

For the evaluation of the pollution load of industrial or domestic wastewaters, a measure of oxygen requirement of pollution matter has been developed as standard parameters, which is known as Biochemical Oxygen Demand (BOD). For the present investigation the BOD level was recorded 240 mg/l initially. Before discharging any industrial effluent the BOD should be removed because it adversely affects the aquatic organisms. In the present study significant removal of both BOD (60.4 %) and COD (51.6 %) was observed. A successful removal of nitrate, nitrite and ammonia was also observed (40 - 50%). The total phosphorus and inorganic and organic phosphate levels were reduced in treated molasses. The results are in par with the similar study done by Chavan *et al*. [14]. Chlorides are generally considered to be one of major pollutant in effluents, which are difficult to be removed by conventional biological methods. In the present study 9-18% removal of chloride from the molasses was observed due to the inoculation of microbes. Similar results were reported by many others [6, 14, 15, 16]. The maximum decolourization of spent wash up to 38% was observed with *P. putida*. This result is supported by the report of Chavan *et al*. [14] who reported 56% decolourization by a *Pseudomonas* sp. *S. cerevisiae* was also able to remove up to 33% of colour from the molasses spent wash. Similar result was reported by Murata *et al*. [17] and Rajor *et al*. [9].The results of present study indicate that both *Pseudomonas putida* and *Saccharomyces cerevisiae*, the local isolates, are efficient in bioremediation of molasses spent wash and can be further exploited for cost-effective, eco-friendly treatment of molasses waste water.

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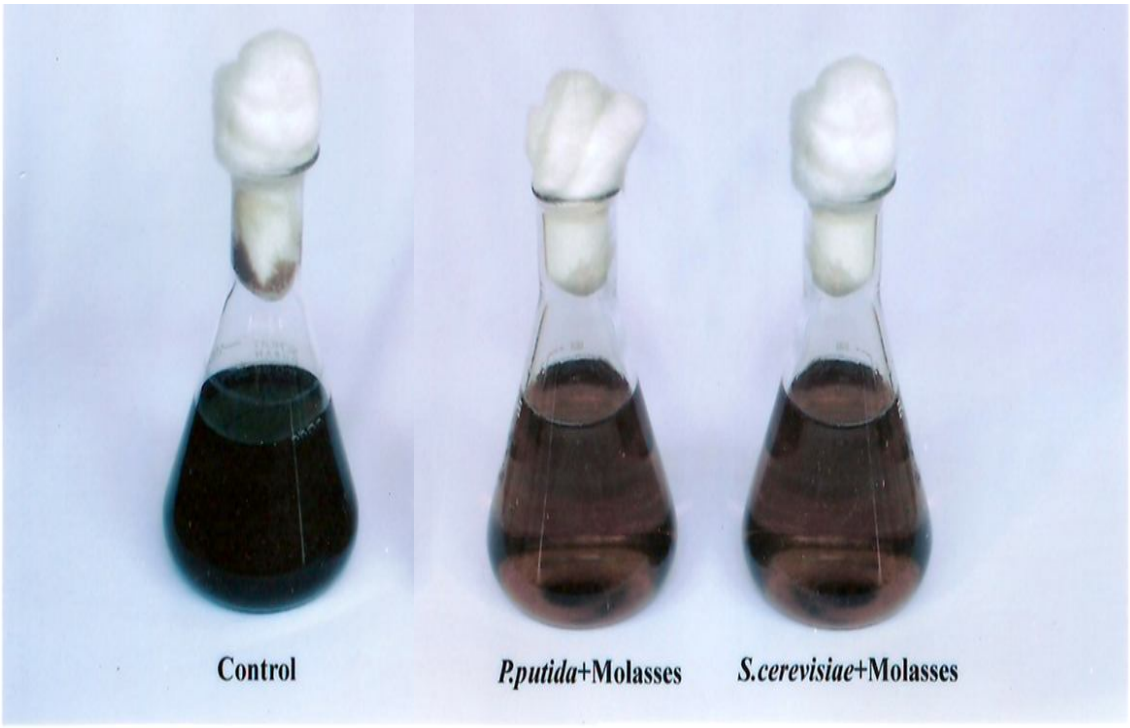

Figure 1. Decolourization of molasses after treating with *Pseudomonas putida* and *Saccharomyces cerevisiae*

Table 1: Biochemical characteristics of isolated bacteria

Sl. No.	Biochemical Tests	*Pseudomonas putida*	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Micrococcus* sp.
1.	Mac Conkey agar test	+	-	-	-	-
2.	Indole test	-	+	-	+	-
3.	Methyl red test	+	+	+	+	+
4.	Voges Proskauer test	+	-	+	-	-
5.	Citrate utilization test	-	-	-	-	-
6.	Starch hydrolysis test	+	-	+	-	+
7.	Urea hydrolysis test	-	-	-	-	-
8.	Nitrate reduction test	-	+	+	+	-
9.	H_2S production test	+	-	+	-	+
10.	Cytochrome oxidase test	+	+	-	+	-
11.	Catalase test	-	+	-	+	-

Table 2. Physicochemical characteristics of molasses spent wash

Sl. No.	Parameters	Control	Treated molasses	
			Saccharomyces cerevisiae	*Pseudomonas putida*
1.	pH*	4.3	6.7	6.7
2.	Free CO_2	31	17	11
4.	DO	1.34	1.45	1.5
5.	Nitrate	90	55	47
6.	Nitrite	48	29	23
7.	Ammonia	42	28	25
8.	Total Phosphate	90	54	49
9.	Inorganic Phosphate	55	31	29
10.	Organic Phosphate	35	23	20
11.	Calcium	64	37	28
12.	Magnesium	37	21	18
13.	BOD	240	114	95
14.	COD	310	180	150

* All values are in mg/l except pH

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RESEARCH ARTICLE

Recent Trends in the Population Status of the Endangered Vulture (*Gyps bengalensis*) Nilgiri District, TamilNadu, India.

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ABSTRACT

The Oriental White backed vulture (*Gyps bengalensis*) once seen widely, but it has now shown decline in its population in many areas. This study was undertaken to collect information about population density of Vultures in different localities like Masinagudi, Theppakadu, Mudumalai and Thengumarahada. The study aims at finding reasons and consequences of changing population pattern of vulture. The study was conducted over a period of one year (October 2010 – September 2011) during which regular field trips were made at intervals of one or two weeks. Population pattern was studied by Road transect and Encounter transect method. It was seen that the vultures inhabit in dense forest like evergreen, moist deciduous and teak forest areas. There is a definite decline in their number over the past few decades. This was because of deforestation, forest fire, loss of nesting sites, food sources, increase in predators and pollution. The majority of dead vultures had visceral gout, due to kidney damage. The realization that Diclofenac, a Non-Steroidal Anti-Inflammatory Drug potentially nephrotoxic to vultures, had become a widely used veterinary medicine led to the identification of Diclofenac poisoning as the cause of the decline. We can conclude that the continual maintenance of multiple widely dispersed provisioning sites it will only ever be possible to reduce Diclofenac contamination. Elimination of Diclofenac in veterinary use is the most certain way to prevent vulture deaths from Diclofenac exposure, although education of veterinarians and livestock owners to avoid treatment of terminally ill livestock, or to bury or burn carcasses of recently treated livestock, may also be helpful.

Keywords: *Gyps bengalensis*, Population status, Nilgiris, Diclofenac, Endangered.

INTRODUCTION

India is characterized with unique richness and diversity of its vegetation and wildlife. India's dense forest reserves are the perfect abode for its equally dense wildlife which includes about 350 mammal species, more than 1200 species of birds in nearly 2100 forms and more than 30,000 species of insects. Besides this, there is rich wealth of marine life having number of species of fish, amphibian and reptiles. More than 75 National parks, 425 Sanctuaries cover over 4.5% of India's geographical area. Indian wild life has got important place in its rich heritage and culture. Many animals and plants are worshipped and are regarded as the companions in India. The country also has about 2000 species and sub-species of birds. The numerous sanctuaries across the country are not only breeding colonies for these feathered creatures, but serve as resorts for migratory birds from higher altitudes, as well. Indian wildlife has its share of native birds along with the migratory birds. Several hundred species of birds can be spotted across India.

Many natural populations have been reduced in size due to increased human activities associated with landscape changes and persecution (Mace *et al.,* 2005). Concern exists because significant reductions in the size of large populations will likely cause a loss of genetic diversity due to drift and increase extinction risk by adversely affecting the ability of populations to adapt to changing environments (Willim *et al.,* 2006). Vulture is the name of a bird that mainly preys on dead animals and occasionally hunts its own quarry. It is found across the world, with the exception of Antarctica and Oceania. The vultures are all obligate scavengers, feeding primarily on the carcasses of large ungulates and nesting and roosting, often colonially, on cliffs or in trees.

The Indian White-rumped vulture (*Gyps bengalensis*) is an Old World vulture in the family Accipitridae. It is closely related to the *Gyps fulvus.* So it was called as Oriental White-Backed Vulture. In the early 20th century, the Indian White-Backed Vulture (*Gyps bengalensis*) was abundant, and its distribution extended from the Thai-Malay peninsula and Indo-china in the east to the Indus River, running through Pakistan, in the west (Oak *et al.,* 2004). However, a recent survey in India indicated a catastrophic collapse in the population of *G. bengalensis*, which had been totally wiped out from 17 key areas in that country (Rae, 1935). *G. bengalensis* was regarded as "possibly the most abundant large bird of prey in the world" (Houston, 1985).

White Rumped Vultures are typical vulture, with an unfeathered head and neck, very broad wings, short tail feathers, medium-sized and dark vultures. It has a white neck ruff. The adult's whitish back, rump and the under wing coverts contrast with the otherwise dark plumage. The body is black and the secondries are silvery grey. The head is tinged in pink and bill is silvery with dark Ceres. The nostril openings are slit-like. Juveniles are largely dark and take about four or five years to acquire the adult plumage. In flight, the adults show a dark leading edge of the wing and have a white-lining on the underside. The under tail coverts are black (Rasmussen and Anderton, 2005).

Adults are 75 to 85 cm tall, their wing span is 180 to 210 cm, and their weight ranges from 3.5 to 7.5 kg. The sexes are approximately equal in size. Adults are darker than juveniles, with blackish plumage, a white neck-ruff, and a white patch of feathers on the lower back and upper tail. During flight, the white under wing coverts are highly visible. Usually the eyes are a yellowish brown colour and the legs are blackish. The bill is short, deep, and stout. Immature *G. bengalensis* are dark brown and the lower back and rump area are brown rather than white. The under wing coverts are dark brown. Eyes are dark brown and the legs are blackish but lighter than the adult. Generally, adults tend towards black colouration, while younger individuals are browner. All *G. bengalensis* can be distinguished by the white bar located on the underside of the wing. (BirdLife International, 2004 & Rasmussen and Anderton, 2005).

According to the *Red Data Book*, Oriental White Backed Vulture has suffered an extremely rapid population decline, particularly across the Indian subcontinent, probably as a result of disease compounded by poisoning, pesticide use and changes in the processing of dead livestock (Threatened Birds of Asia, 2001).

MATERIALS AND METHODS

The present study was taken to reveal the population capacity and addition of the Meloxicam drug an alternative for the Diclofenac toxicity of endangered vulture, *Gyps bengalensis* (Plate-1). The study was made in Nilgiri district, Tamilnadu, India.

Study area
Mudumalai sanctuary

The Mudumalai Sanctuary is as an important wildlife habitat, because of its strategic position. As a Wildlife corridor between several other protected areas that are a part of the Nilgiri Biosphere Reserve. At north to this the Bandipur National Park and Nagarhole National Park is there and in the west the Wayanad Wildlife Sanctuary and in the south Mukurthi National Park and Silent Valley National Park are there. At the east the Segur plateau which connects to the Sathyamangalam wildlife sanctuary and Reserve forests is located. There is a high diversity of animal life in the sanctuary with about 50 species of fishes, 21 species of amphibians, 34 species of reptiles, 227 species of birds and 55 species of mammal. Mammal diversity is higher in the dry deciduous and dry thorn forests than in the other habitats. Thirteen percent of all mammal species in India are present in Mudumalai wildlife Sanctuary. Eight percent of bird species in India occurring in Mudumalai Wildlife Sanctuary. Among the 227 bird species found in Mudumalai, 110 species are insectivores, 62 are carnivores, 23 species are fishivores, 12 species are omnivores and 20 species are grainivores.

Thengumarahada
Thengumarahada occupy the area of 28 sq.km and 9.4km away from Kotagiri taluk.

Climatic conditions

In Mudumalai sanctuary the winter season takes place from November to February. Normally it has the temperature from 18º C -20º C and average rainfall 1020mm. The hot summer season falls at March to May and the temperature ranges from 26º C -33º C, average rainfall 790mm and the rainy monsoon season usually arrives at June to September. This season has heavy rainfall 1840mm and the temperature ranges from 20ºC -24ºC. In Thengumarahada the winter season are from November to February. It has the temperature 20º C -22º C and average rainfall 870 mm. The hot summer season are from March to May and the temperature ranges from 28º C -32º C, average rainfall 640 mm and the rainy monsoon season usually from June to September. In this season has heavy rainfall 1450 mm and the temperature ranges from 20º C -24º C.

Catchment areas

The studies were done at two locations in Nilgiris district. They are Mudumalai sanctuary (**Masinagudi, Thepakadu, Mudumalai**) and Thengumarahada. The sanctuary has an area of 321Sq. Km with 108 Sq.km of National park area. The altitude ranges from 100' MSL to 1200' MSL. Thengumarahada occupy the area of 28 sq.km and 9.4km away from Kotagiri taluk.

Road transect method (Fuller and Mosher, 1987)

Road transect method is very useful for counting vulture ranging in wider areas. This method was employed to determine vulture species distribution in Mudumalai sanctuary and Thengumarahada. Roads and trails in the park provided convenient access to the study area were used as transects for survey carried out monthly in clear weather by a two member team about an hour after sunrise on a moving vehicle (with speed 20 km/hr) stopped at specified points to survey the vulture. This allowed large areas of the park to be searched efficiently for vulture. A pair of good binoculars and digital camera was used to aid identification of distant birds.

Encounter transects (Abdul Jamil Urfi, 2004)

Using prepared base map identifies (preferably randomly) the starting point of the transect, before starting our survey record the required transect information and weather conditions at the top of the data form: Bird Observation Form – Vulture.This method was employed to determine vulture species distribution in Mudumalai sanctuary and Thengumarahada. 0.5-2 Km are recorded as one transect, like that 23 transects were recorded in the survey area. Among these 19 transects in Mudumalai and 4 transects in Thengumarahada were studied. For each vulture detection, record the distance traversed along the transect. Vultures encountered along the transect must be recorded. Each transect should be surveyed in morning to evening.

Visual Scanning Method (Jathar and Rahmani, 2003)

The field survey for the vulture was carried out by the visual scanning method. Visual scanning involves purposeful scanning of treetops for locating the vultures. For this purpose, a binocular was used. Field trials for this method were taken to assess best times of the day for visual scanning. Early to mid-mornings and evenings were found to be the best times.

Data Collection Parameters
Macro and Microhabitat Assessment

From previous studies and literature, it was known that the vulture prefers dense forests and teak-bearing miscellaneous forests and can also be found in areas close to human habitation. We recorded a number of survey parameters at each survey point to determine the habitat preference of the species. We also carried out an assessment of threat due to human presence in the area. These are described below.

Field Survey Protocol
Composition of the Survey Team

The survey teams consisted of a field biologist and a field assistant and were generally accompanied by a guide, either a local villager or a staff member of the Forest Department. Presence of a staff member during the survey was useful in obtaining information about the area, location and compartment numbers of the site.

Selection of Survey Locations

Forest divisions having good quality vegetation cover were selected from the vegetation density maps of each state prepared by Forest Survey of India (Forest Survey of India, 2005). Forest Divisions having fragmented and low density forests were not included in the survey. Based on the premise that the vulture was found in ever green forests

we selected forest circles and forest divisions having evergreen forests and moist deciduous forests, for the survey. These divisions were selected based on discussions with knowledgeable forest officers of the state.

Survey Itinerary

The route for the survey was planned prior to starting the survey. From the vegetation map of the state, districts having open dry deciduous teak forests were selected and marked on the map. Survey itinerary was finalized after assessing the area to be covered and the available time.

Discussion with Local Officers and Staff

Before beginning the survey in a forest division the survey team generally met the Deputy Conservator of Forests of the forest division and obtained information regarding the best ranges to survey. Ranges having fragmented and degraded forests were omitted. Ranges having open dry deciduous teak forests were selected for the survey. With the help of the range forest officer and field staff of the range we identified convenient roads and trails passing through the forests of that range for carrying out the survey. The survey was carried out in the forests along these roads and trails.

Season

Seasonal effects can be more difficult to cope with. Bird conspicuousness will probably change with season, and in tropical forests there may not be synchronization of breeding cycles between or even within species. In a species which is breeding, the males may be singing and calling to defend a territory and so may be easy to record, whereas the females incubating eggs may be the opposite. There can be no hard and fast rules about whether studies are better designed to avoid or coincide with the peaks of breeding activity, as this is better determined by the aims of the study.

Weather conditions

Adverse weather conditions such as low cloud, high winds, rainfall and even very high temperatures can affect census results in three ways. Firstly, bird activity can be directly affected (usually reduced), which will affect the efficiency and reliability of our data collection. Secondly, the conditions could reduce our chances of actually seeing or hearing the birds. Thirdly, we cannot pay adequate attention to counting if the conditions are too hot, too cold or wet. Census results can also be affected by conditions underfoot (during dry periods, fallen leaves may become very noisy to walk on), or by the noise of cicadas (whose activity is influenced, amongst other things, by temperature and humidity).

RESULTS

The survey areas are Masinagudi, Theppakadu, Mudumalai and Thengumarahada. The survey took place in the three different seasons, from October to September. Survey of vultures done in winter, hot summer and rainy monsoon seasons. In Mudumalai sanctuary winter season, temperature is very low at 18ºC to 20 ºC, hot summer season, temperature is very high at 33 ºC and in rainy monsoon season the temperature come to down at 22 ºC to 24 ºC. At end of the winter season only (Nearly February) the vultures can be seen frequently. In hot summer seasons the vultures were seen rarely because of high temperature, scarcity of food, water and mainly forest fire. In rainy monsoon season there will be heavy rainfall, it affects our survey and also birds were migrated to another place. In

Thengumarahada winter season, temperature is very low at 20ºC to 22 ºC, hot summer season, temperature is very high at 28ºC to 32ºC and in rainy monsoon season the temperature come to down at 20 ºC to 24 ºC (Table 1 and Fig, 1, 2).In Mudumalai sanctuary, highest rainfall 1840 mm in rainy monsoon season. In winter season 1020 mm rainfall and in summer season 790 mm rainfall was noticed. In Thengumarahada highest rainfall 1450 mm recorded in rainy monsoon season. In winter 870mm and hot summer 640 mm rainfall was registered (Table 2 and Fig. 3, 4).

In our survey most vultures are recorded in Mudumalai than other three regions. Only one vulture was observed in Thengumarahada (Fig. 5). The survey has done by two different methods namely Road transect method and Encounter method. Road transect method done by moving vehicle. In road transect method, each transects are separated based on the kilometers. The study area was divided into nine transects. Each transects contain 10 Sq. Km. In road transect method, only three individual vulture were found in Theppakadu, Mudumalai and Thengumarahada. In this project there is no possibility to see the vultures in Masinagudi area in road transect method. Vultures seemed by road transect method in Theppakadu at 30-40 km ranges, in Mudumalai at 70-80 km ranges and in Thengumarahada at 10-20 km ranges (Table 3, Fig. 7).

Encounter transect method was done in same study area for counting the vulture population this method was very convenient. In encounter method each and every Sq. km surveyed. So this method is very accurate. Each transect was occupied every Sq. Km. of the areas. In Masinagudi, three vultures are observed in February and June (Table 4a). In Theppakadu and Thengumarahada three vultures was recorded in February and March in dense forest areas (Table 4 b and 4d). The rich flora and fauna were found in Mudumalai area (Table 4c). The availability of food is more in this area. In this area only high numbers of vultures are recorded in January, February and July. In both of the transect methods, more number of vultures were recorded by Encounter transect method (Fig. 7).

In total, we covered 132 Sq. Km. across 89 Sq. Km. for road transect method and 43 Sq. Km. for encounter transect method. There, we saw 11 individuals of white- backed vultures in various habitats. Our study areas are rich in vegetation. So vultures are breed in those areas. In that nine white-backed vultures are found exclusively in the evergreen and moist deciduous forest. Other two individuals sited in teak forest. Evergreen forest contains high number of large trees. It is a good habitat for vultures, because vultures want to live in highest places (Table 5, Fig. 6).

DISCUSSION

In South Asia, the populations of the endemic Oriental White Backed (*Gyps bengalensis*), Slender Billed (*Gyps tenuirostris*) and Long Billed (*Gyps indicus*) vultures have declined dramatically by more than 95% since the early 1990s, and the evidence suggests that the few that are left continue to decline at between 15% and 50% per year. These three species which, together, just 15 years ago, used to number tens of millions (possibly 40 million of one species in India alone), are now almost unbelievably at serious risk of global extinction and are listed as 'Critically Endangered' by the International Union for Conservation of Nature (IUCN, 2005).

The decline of vultures is worrying from a biodiversity viewpoint, but also because a great number of carcasses are being left uneaten" Cunningham (2000) says, "Vultures play an important role in scavenging human and animal carcasses". For example, In India's 76000 strong Parsees community peoples bones do not buried but their bones are cleaned by vultures alone. In India, Gyps vulture's numbers diminished by several reasons. Extreme examples are *G. bengalensis*, which often lives in close association with humans. Humans may be a threat to *G. bengalensis* because of the usage of vultures as a source of medicines (Aminur Rahman, 2009), diseases, pesticides (DDT and HCH), environmental contamination, poisoning, habitat alterations, reduced food availability (Thiollay, 1998), calcium

deficiency, reduced nesting habitat, nest predators, hunting (Pain *et al.,* 2008), Climate change (Mc carty, 2001) and aircraft strikes (Bhatnagar, 1993).

Diclofenac is a widely available veterinary drug in the Indian subcontinent, where it is used for the symptomatic treatment and management of inflammation, fever, and/or pain associated with disease or injury in domestic livestock. Scientific evidence following the observation of waste disposal practices of carcasses confirms without doubt that the veterinary use of Diclofenac is the main cause of these declines (Green *et al.,* 2004) throughout the Indian subcontinent.

The realization that Diclofenac, a Non Steroidal Anti-Inflammatory Drug potentially nephrotoxic to birds, had become a widely used veterinary medicine led to the identification of Diclofenac poisoning as the cause of the decline. Surveys of Diclofenac contamination of domestic ungulate carcasses, combined with vulture population modelling, show that the level of contamination is sufficient for it to be the sole cause of the decline. Post-mortem examinations of vulture carcasses revealed visceral gout (deposition of uric acid crystals in the tissues) as the cause of mortality in most of the dead birds.

As a consequence of the collapse of South Asian vulture populations, national and international conservation organizations have concluded that it is essential to ban the use of Diclofenac in domestic livestock so as to remove it as a toxic contaminant of the food of wild, scavenging vultures. At a meeting of the National Wildlife Board in March 2005, the Government of India announced that it intended to phase out the veterinary use of Diclofenac within six months. In 2006, the Governments of India banned the manufacture of Diclofenac. This sends a very clear signal and is welcome. However, retail sale of Diclofenac not manufactured for veterinary use remains legal in these countries, so full bans on retail sale for veterinary use may be necessary (Nita Shah, 2008).

CONCLUSION

The Governments of the respective countries may take immediate steps to completely phase out veterinary Diclofenac. This would have to be complemented by aggressive awareness campaigns about the adverse ecological effects of the drug. To conduct a reliable population estimate at State, country and regional levels.

Efforts should also be made to raise strengthen education and awareness campaigns among veterinarians, pharmacists, livestock owners and general public, of the problem of Diclofenac contamination and availability of safe alternative Meloxicam. Captive holding and breeding of vultures until Diclofenac is controlled is recommended as a precaution to ensure the long term survival of the threatened species and to provide a stock of birds for vultures reintroduction programme.

In situ and *Ex situ* conservation project, vultures should be fed on carcasses of animals that are known not to have been treated with Diclofenac in the week before death. Vulture restaurant will be a great solution for increasing the population of *Gyps bengalensis* to be with poisonless feeds. It will help the vultures from poison death and will increase in its genes.Setting up of a Task Force for the region to collaborate in vulture conservation programmes in these regions.

Table 1: Temperature and seasons of the study areas

Survey area	Month	Season	Temperature (ºC)
Mudumalai sanctuary a) Masinagudi b) Thepakadu c) Mudumalai	October-February	Winter	18 ºC- 20 ºC
	March-May	Hot summer	26 ºC- 33 ºC
	June-August	Rainy monsoon	20 ºC- 24 ºC
Thengumarahada	November-February	Winter	20 ºC-22 ºC
	March-May	Hot summer	28 ºC-32 ºC
	June-September	Rainy monsoon	20 ºC-24 ºC

Table 2: Rainfall and seasons of the study areas

Survey area	Month	Season	Rainfall (mm)
Mudumalai sanctuary a) Masinagudi b) Thepakadu c) Mudumalai	October-February	Winter	1020
	March-May	Hot summer	790
	June-August	Rainy monsoon	1840
Thengumarahada	November -February	Winter	870
	March-May	Hot summer	640
	June-September	Rainy monsoon	1450

Table 3: Vultures observed in study areas by Road transect method

Survey area	No. of Vultures observed in road transect (Sq. km)								
	1-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90
Mudumalai sanctuary a) Masinagudi	-	-	-	-	-	-	-	-	-
b) Theppakadu	-	-	-	**	-	-	-	-	-
c) Mudumalai	-	-	-	-	-	-	-	**	-
Thengumarahada	-	**	-	-	-	-	-	-	-

****** Observed, -Not Observed

Table 4a: Vultures observed in study area by Encounter transect method

Survey area	Survey Month	No. of Vultures observed in Encounter transect												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Masinagudi	**October**	-	-	-	-	-	-	-	-	-	-	-	-	-
	November	-	-	-	-	-	-	-	-	-	-	-	-	-
	December	-	-	-	-	-	-	-	-	-	-	-	-	-
	January	-	-	-	-	-	-	-	-	-	-	-	-	-
	February	-	-	**	**	-	-	-	-	-	-	-	-	-
	March	-	-	-	-	-	-	-	-	-	-	-	-	-
	April	-	-	-	-	-	-	-	-	-	-	-	-	-
	May	-	-	-	-	-	-	-	-	-	-	-	-	-
	June	-	-	-	-	-	-	-	-	-	**	-	-	-
	July	-	-	-	-	-	-	-	-	-	-	-	-	-
	August	-	-	-	-	-	-	-	-	-	-	-	-	-
	September	-	-	-	-	-	-	-	-	-	-	-	-	-

****** Observed, -Not Observed

Table 4b: Vultures observed in study area by Encounter transect method

Survey area	Survey Month	No. of Vultures observed in Encounter transect			
		1	2	3	4
Theppakadu	October	-	-	-	-
	November	-	-	-	-
	December	-	-	-	-
	January	-	-	-	-
	February	**	-	-	-
	March	-	-	**	-
	April	-	-	-	-
	May	-	-	-	-
	June	-	-	-	-
	July	-	-	-	-
	August	-	-	-	-
	September	-	-	-	-

******Observed, -Not Observed

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RESEARCH ARTICLE

Importance of biodiesel fuel in Rural North Karnataka region,Karnataka, India.

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ABSTRACT

The scarcity of known petroleum reserves will make renewable energy resources more attractive. The most feasible way to meet this growing demand is by utilizing alternative fuels. Biodiesel is defined as the monoalkyl esters of vegetable oils or animal fats. The biggest advantage that biodiesel has over gasoline and petroleum diesel is its environmental friendliness. Biodiesel burns similar to petroleum diesel and probably has better efficiency than gasoline. It has over double the price of petrodiesel. The major economic factor to consider for input costs of biodiesel production is the feedstock, which is about 80% of the total operating cost. The high price of biodiesel is in large part due to the high price of the feedstock. Since, India has been actively promoting the cultivation of *Jatropha* on unproductive and degraded lands for the production of biodiesel suitable as transportation fuel. Jatropha are known just crude plants which grow on eroded soils and require a hot climate and hardly any water to survive. These are the strong reasons, enforcing the development of biodiesel plants. In this paper the life cycle energy balance, global warming potential and land use impact on ecosystem quality is evaluated for a small scale, low-input *Jatropha* biodiesel system established on wasteland in rural India.

Keywords: Biogas; Energy; *Jatropha curcas*; Waste Land; fossil fuel; fertilizers.

INTRODUCTION

Biodiesel is an alternative to conventional diesel fuel made from renewable resources, such as non-edible vegetable oils. The oil from seeds (e.g., Jatropha and Pongamia) can be converted to a fuel commonly referred to as "Biodiesel." No engine modifications are required to use biodiesel in place of petroleum-based diesel. Biodiesel can be mixed with petroleum-based diesel in any proportion. This interest is based on a number of properties of biodiesel including the fact that it is produced from a renewable domestic source, its biodegradability, and its potential to reduce exhaust emissions. The climate change is presently an important element of energy use and development. Biodiesel is considered "climate neutral" because all of the carbon dioxide released during consumption had been sequestered out of the atmosphere during crop growth. The use of biodiesel resulted in lower emissions of unburned hydrocarbons, carbon monoxide, and particulate matter

Biofuel development in India centers mainly around the cultivation and processing of Jatropha plant seeds which are very rich in oil (40%) (Michael Whitaker). The drivers for this are historic, functional, economic, environmental, moral and political. Jatropha oil has been used in India for several decades as biodiesel for the diesel fuel requirements of remote rural and forest communities; jatropha oil can be used directly after extraction (i.e. without refining) in diesel generators and engines.(Archana Joshi.et.al) Jatropha has the potential to provide economic benefits at the local level since under suitable management it has the potential to grow in dry marginal non-agricultural lands, thereby allowing villagers and farmers to leverage non-farm land for income generation. since Jatropha oil is carbon-neutral, large-scale production will improve the country's carbon emissions profile. Finally, since no food producing farmland is required for producing this biofuel (unlike corn or sugar cane ethanol, or palm oil diesel), it is considered the most politically and morally acceptable choice among India's current biofuel options; it has no known negative impact on the production of the massive amounts grains and other vital agriculture goods India produces to meet the food requirements of its massive population (circa 1.1 Billion people as of 2008). Other biofuels which displace food crops from viable agricultural land such as corn ethanol or palm biodiesel have caused serious price increases for basic food grains and edible oils in other countries.

India's total biodiesel requirement is projected to grow to 3.6 Million Metric Tons in 2011-12, with the positive performance of the domestic automobile industry.(Frost & Sullivan,) Analysis from Frost & Sullivan, *Strategic Analysis of the Indian Biofuels Industry*, reveals that the market is an emerging one and has a long way to go before it catches up with global competitors.The Government is currently implementing an ethanol-blending program and considering initiatives in the form of mandates for biodiesel.

Biodiesel is now being produced for the use in three-wheeler motor rickshaws. These engines actually run on regular diesel fuel or CNG, but in the past kerosene were used because it was far cheaper, and worked just as well. However, kerosene was dirty and wasn't as clean-burning. Biodiesel is rapidly replacing both kerosene and diesel as a more efficient, cheap, and clean alternative. Now plans are being chalked out to cultivate jatropha plants on barren land to use its oil for biodiesel production. Now it is used for railway engines and the plantations are recommended to plant these plants everywhere in unused areas through government sectors. Biodiesel is being used experimentally to run state transport corporation buses in Karnataka. Large scale activities have been initiated quite recently. For example, large-scale plantations have been initiated in North-East India and Jharkhand by D1 Williamson Magor Bio Fuel Limited, a joint venture between D1 Oils of U.K. and Williamson Magor of India. The hilly areas of the North-East are ideal for growing this hardy, low-maintenance plant. (Fig:2)

MATERIALS AND METHODS

Study area

Gulbarga district lies in the northern part of Karnataka between 16°11' –17°45' N. latitudes and 76°03' - 77°30' E. longitudes, with a geographical area of 16,174 sq. km. Gulbarga is one of the constantly drought prone district in North Karnataka. The district is bounded by Bidar district in the north, Bijapur district in west, Raichur district in south and Andhra Pradesh in the east. The Average Annual Rainfall (mm) 832.30. Gulbarga is the district headquarters. The district comprises of 10 taluks namely, Aland, Afzalpur, Gulbarga, Chincholi, Chittapur, Sedam, Jewargi, Shahapur, Yadgiri and Shorapur. There are 48 hoblies, 337 Gram Panchayats, 12 Municipalities, 18 Towns/ Urban agglomeration and 1360 inhabited & 77 un-inhabited villages (Fig.1).

Irrigation practices

About 75% of the geographical area of the district is under cultivation. Irrigation through dug wells is more prevalent in Aland, Gulbarga, Yadgiri & Afzalpur taluks, whereas, irrigation in Shorapur, Shahpur and Jewargi taluks is through canal of Upper Krishna Project. Lift Irrigation Schemes are under implementation along Bhima River.

Rainfall & Climate

The southwest monsoon sets in the middle of June and extends till the end of September. Bulk of the annual rainfall occurs during this season, which constitutes over 75% of the annual rainfall. Significant rainfall occurs during the winter monsoon owing to northeastern monsoon, which constitutes 15% of the annual rainfall. Normal Rainfall of the district is 777 mm (1901 - 70) and actual rainfall is 881.10 mm (2005). Normal rainy days (as per 1901 - 70) are 46. Although, consistent normal rainfall is prevalent, Sedam, Chincholi & Chitapur taluks experience mild drought conditions. (Ministry of Water Resources-Gulbarga District, Govt of India-2008).Gulbarga district lies in the northern plains of Karnataka and has semi – arid type of climate. Dry climate prevails for most part of the year. December is the coldest month with mean daily maximum and minimum temperatures being 29.5°C & 15° to 10°C respectively. During peak summer, temperature shoots

The area comes under irrigated region with moderately high temperature. The prominent agricultural crops of this region include paddy, Jowar, Bajra, Gram, Turi, Groundnut and Sunflower. Oil expel unit is present in Gulbarga University campus. And the oil obtained from biofuel plants are using instead of diesel and also for various other purpose. The waste obtained from extraction of oil is used as manure in farms.

Major soil types are, Deep black soil, Medium black soil & lateritic soil

The survey was conducted by selecting Two sites in Gulbarga taluks, One of them representing irrigated (i.e Jevargi) and other representing dry regions of the taluk (Yadgiri). The sites were selected on the basis of density of the plant species, rainfall and pattern of irrigation and soil characteristics of the regions. For the purpose laboratory analysis and the details of seed samples were tabulated. The survey was conducted in the Months of Oct- December- 2010. During the study period primary information about the density of *Jatropha curcas* was collected . In each location,

mature pods from ten healthy trees species were collected. Seed weight was determined with a sample of 100 seeds. Number of pods and seeds per kg were also calculated. The fruits pods were split open and the seeds were separated. Seed length, breadth and thickness were measured with digital vernier calipers.

The wasteland area available in Karnataka is 788017 ha of wasteland (Source: State land use board. Bangalore**).** Tumkur (Southern Karnataka) has the highest area of 67539 ha of wasteland while the study area (Gulbarga) has 16372 ha of Wasteland (Source: State land use Board. Bangalore). Bio-fuel plants can be grown in at least 50% of the wasteland namely 5686 ha in the study area. The Central Government has approved mixing of 10% bio-fuel with diesel in cars, whereas there are experiences in case of trucks and tractors, mini vans with higher percentages of bio-fuel use in India.

Description of Tested Plants

There are many tree species which bear seeds rich in oil having properties of excellent fuel and which can be possesses into diesel substitute. In Karnataka the important commercial non-edible oil yielding plants are, *Pongamia pinnata, Jatropha curcas, Azadirachta indica, Madhuca longifolia ,Schlichera oleosa, Garcinia indica , Calophyllum inophyllum* and many more. The oil obtained from such seeds is chiefly used for manufacture of soaps, candles, paints, varnishes, linoleum, and lighting for medicinal purposes. Among the various oil yielding plants *Jatropha curcas* were selected for the study. (Fig:3)

Jatropha curcas is a species of flowering plant in the spurge family, Euphorbiaceae that is native to the American tropics, most likely Mexico and Central America. It is cultivated in tropical and subtropical regions around the world. Common names include Barbados Nut, Purging Nut or Physic Nut. *J. curcas* is a poisonous, semi-evergreen shrub or small tree, reaching a height of 6 m (20 ft). It is resistant to a high degree of aridity, allowing it to be grown in deserts.

Seeds: The seeds contain 27-40% oil (average: 34.4%) that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine. The seeds are also a source of the highly poisonous toxalbumin curcin. The seeds are mature when the capsule changes from green to yellow. The seeds contain around 20% saturated fatty acids and 80% unsaturated fatty acids, and they yield 25%–40% oil by weight. In addition, the seeds contain other chemical compounds, such as Saccharose, Raffinose, Stachyose, Glucose, fructose, Galactose, and Protein. The oil is largely made up of oleic and linoleic acids. Furthermore, the plant also contains curcasin, arachidic, linoleic, myristic, oleic, palmitic, and stearic acids and curcin.

Leaves: The leaves have significant variability in their morphology. In general, the leaves are green to pale green, alternate to sub opposite, and three- to five-lobed with a spiral phyllotaxis. Male and female flowers are produced on the same inflorescence, averaging 20 male flowers to each female flower, or 10 male flowers to each female flower. The petiole length ranges from 0.24 to 0.90 inches (6.1–23.1 mm). The inflorescence can be formed in the leaf axil. Plants are monoecious and also present hermaphroditic flowers occasionally.

Fruits: Fruits are produced in winter, or there may be several crops during the year if soil moisture is good and temperatures are sufficiently high. Most fruit production is concentrated from midsummer to late fall with variations in production peaks where some plants have two or three harvests and some produce continuously through the season.

RESULTS AND DISCUSSION

Highest seed weight (100 seeds) of *J.curcas* was in the collection at Jevargi region, the lowest was noticed from Yadgiri region. Thus, the seed weight was more in Jevargi and lowest in Yadgiri. In case of seed length, Jevargi region witnessed highest seed length, while seed length was lowest at Yadgiri region. And the Seed breadth was noticed highest in Jevargi . While the, lowest among the collected seed samples was witnessed by Yadgiri. According to the study,

There were significant differences noticed during the study period among the seed characteristics of the tested plant species at study area. Variation of seed sources with respect to their morphological characters of seed and seedlings could be done to the fact that this species grows over a wide range of climatic conditions. From the time immemorial, plants have been widely used as curative agents for variety of ailments. Concentrated fruits or seeds extract can be found in various herbal preparations are widely available in market today. *Jatropha curcas* oil is widely available and employed by practitioner of natural health for treatment of rheumatism. In the traditional systems of medicines, such as Ayurveda and Unani, Its oil is a source of biodiesel. It has also alternative source of energy, which is renewable, safe and non-pollutant.

In general the small-scale *Jatropha* biodiesel system for local transportation use shows similar environmental performance as other biofuel systems. Compared to other systems our case study shows a strong reduction in non-renewable energy requirement and a moderate reduction in global warming potential. The trade-off environmental cost for these reductions is an increase in eutrophication and acidification. Expanding the *Jatropha* biodiesel system with biogas production enhances the energy efficiency while other impacts remain stable due to other offsetting factors. As fertilizer and waste applications (mainly N) are important contributors in most impact categories, optimizing fertilization and agronomic practices and improving crop uniformity through breeding are seen as the major system improvement options, along with the efficient use of by-products and technological advances.

So that if we grow this plants in the waste lands regions it could helps in the future. There is clear indication that *Jatropha* cultivation can make a significant contribution to the bio-fuel production and in sustainable development of the country, Although this *Jatropha* system for local use shows some promising LCA results, it has to be noted that these reflect environmental performance and not complete sustainability. The study does not consider socio-economic impacts. Even though the *Jatropha* cultivation on wasteland does not trigger direct (or even indirect) competition with food and is expected to create only low carbon debt it might still compete with other resources (e.g. labour, water, etc.). However a small scale, low input system creates income generation opportunities. Hence, this study is a partial contribution to increase insight in the sustainability potential of *Jatropha* based biodiesel systems.

Moreover, due to uncertain supplies and fluctuations in prices for fossil fuel in international market, the need to search renewable, safe and nonpolluting sources of energy assumes top priority. Non-edible oil bearing trees like *Jatropha* and karanja can be utilized either as biofuel or with processing. The use of these trees on wastelands is of vital importance for the human population in developing countries. Biodiesel has because it is environmentally safe can be made from renewable sources and prepared locally. Since India is deficient in edible oils, the non-edible oil like karanja, *Jatropha*, etc could be the desirable source for India for production of bio diesel. These plants could be grown on wasteland, about 80 million hectare of which is available in India. These crops grow in arid and semi-arid region and require almost no post plantation management and care. Since, almost all the wasteland is available in rural and economically underdeveloped region, the large-scale bio diesel production has an enormous potential for employment and development of these areas.

CONCLUSION

As a substitute for fast depleting fossil fuel. Bio diesel had come to stay. In future, it should also serve to reduce and maintain the price of automobile fuel. The under exploited and un exploited vegetable oils are good sources of biofuel. Our country is endowed with many such plants. Research is being carried out now to convert vegetable oils into biodiesel through biotechnological processes using biodiesel. With a concentrated and coordinated effort. Wide use of bio diesel in our country is going to be a reality in the days to come.

A national mission on Bio-Diesel has already been proposed by the committee comprising six micro missions covering all aspects of plantation, procurement of seed, extraction of oil, trans-esterification, blending & trade, and research and development. Diesel forms nearly 40% of the energy consumed in the form of hydrocarbon fuels, and its demand is estimated at 40 million tons.Therefore blending becomes the important National Issue which apart from giving the dividends, it saves the country's exchequer. India has vast stretches of degraded land, mostly in areas with adverse agro- climatic conditions, where species of Jatropha, Mahua etc can be grown easily.

Even 30 million hectares planted for bio- diesel can completely replace the current use of biofuels. The production of Bio fuels will also boost the rural economy which will bring more enthusiasm in more than one billion lives in the area. India produces about 25% and imports 75% of its oil requirement. India is the least explored region for oil. India's import bill is about 100,000 cores per annum and consumption is about 2% of world's oil. *Jatropha* is a perennial tree living for 40 to 50 years or more depending upon local conditions. The *Jatropha* takes about 2 to 3 years to commence fruiting and another 2 years or more to come to the stage of full bearing. Thus, only after 4 or 5 years will the grower be in a position to reap the reward for his labours. If the original planting material used happens to be poor in quality, it will result in the establishment of a plantation giving poor yields and turn out to be a source of loss to the grower as long as the plantation lasts. He has no other option but to uproot the unproductive and uneconomic trees. These facts will underline the need to plant quality planting material that will ultimately give good yields. The *Jatropha* is capable of being propagated by seed and cuttings. As tree is cross-pollinated in nature, the progeny is likely to be variable, but certain precautions, if observed, are found to help in the production of quality planting material. The *Jatropha* crops have been recommended for cultivation in the wasteland so that the cultivated lands may not be diverted for *Jatropha* crop. *Jatropha* being the drought tolerant and hardy crop, it can be grown under various kinds of wasteland, however, being the oil yielding plant, it also needs the fertile soil for its cultivation and regular bearing of fruits with desired oil content. Though, the plant can survive in the stress condition, however, it will not give economical yield.

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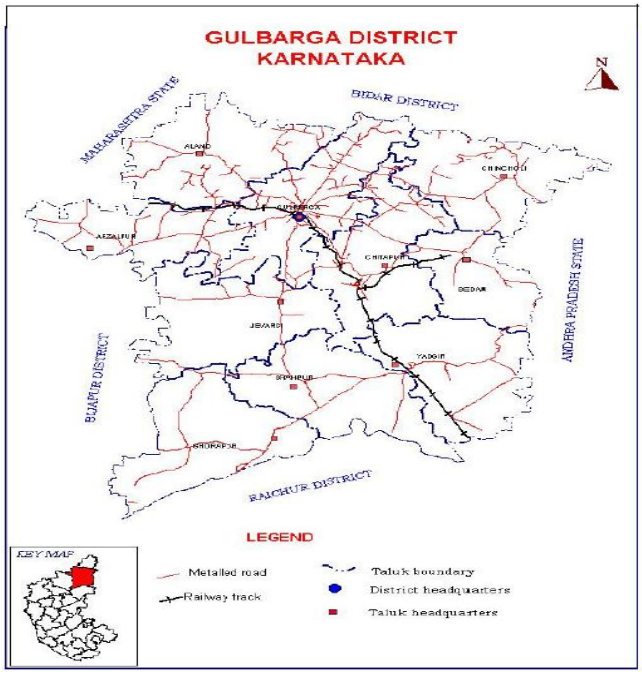

Fig.1.Study Area

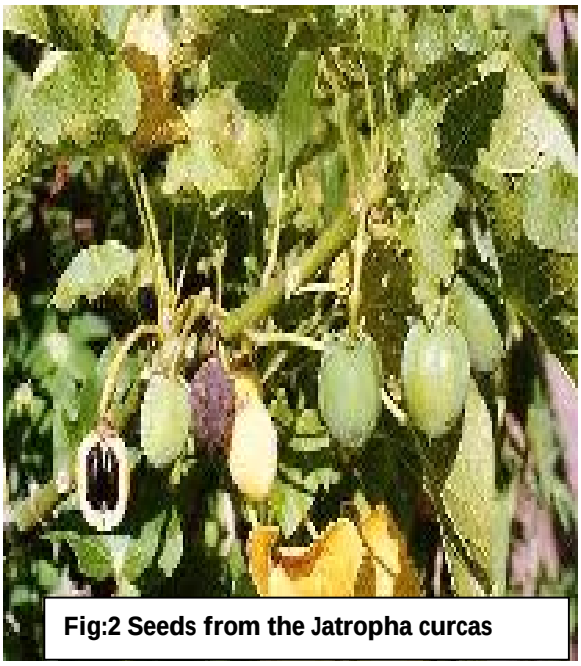

Fig:2 Seeds from the Jatropha curcas

Fig.3 . *Jatropha curcas* plantation in the Study area.

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RESEARCH ARTICLE

An Enumeration of Different Organic and Inorganic Manure on Transplanted Rice (KMP-101TANU)

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ABSTRACT

A field experiment have been carried out to study the impact of different combination of organic sources (Vermicompost, FYM and inorganic sources of plant nutrients) and individual application of organic manures on the performance of transplanted rice variety KMP-101 (TANU). Vermicompost at 10t/ha when combined with Recommended Dose of Fertilizer produced significantly higher plant height and more number of tillers/plant as observed compared to Recommended Dose of Fertilizer alone and vermicompost applied at 5t/ha. Similar trend was also noticed in case of yield attributes viz. number of panicles/plant, filled grains/panicles, grain yield/ha. Application of vermicompost at 15t/ha also produced significantly higher number of growth components viz. tillers/plant, filled grains/panicle, grain yield/h as compared to no application of fertilizer, 100% of Recommended Dose of Fertilizer and FYM at 10/ha. But it was on par with the treatment that received 100% of Recommended Dose of Fertilizer +Vermicompost at 10/ha. Thus the results indicated that besides sustaining the fertility status of the soil, higher grain yield of rice could be achieved when 10t/ha vermicompost applied along with Recommended Dose of Fertilizer or vermicompost alone applied at 15/ha as compared with Recommended Dose of Fertilizer alone.

Keywords: Vermicompost, Rice, Farmyard Manure, Recommended Dose of Fertilizer.

INTRODUCTION

Sustainable agriculture emphasizes the conservation or its own resources to produce adequate amounts of high quality foods but agricultural research in the post green revolution period concentrated entirely on increasing the efficiency of intensive external input for higher crop productivity. The agricultural technologies develop as part of these research efforts have left considerable degradation of resources both to the soil and the environment understating the consequences in a global perspective the current research efforts addressed the sustainability issues incorporating environmental friendly technologies.In the field of agronomy development of integrated nutrient management practices, which ensures optimal mix of organic and inorganic fertilizer has emerged as a major field of research. In many occasions complete organic farming oriented concepts have also been witnessed.

Rice based cropping system occupies 9.7 million ha in India. World rice production in 2004 was 610 m.t cultivated in an area of 147 m.ha with the productivity of 3.75 t/ha .The productivity of Rice in India as low as that of world average which is mainly due to existing low yielding varieties and susceptible to pests and diseases and apart from a suitable nutrient management influence the production. In addition to this balanced fertilization also necessary for rice crop along with soil testing for attaining maximum yield, maximum profit and desired yield targets.

Continuous and unbalanced use of inorganic fertilizer leads to decline or stagnation in productivity due to limitation of one or more nutrients. The results of long term fertilizer experiment revealed that sustainable production of rice could be achieved only by maintaining a balance between supplied and demand of nutrient by integration of in organic and organic sources of nutrient like farmyard manure / vermi compost and chemical fertilizer. The present investigation was therefore taken up to study the effect of various organic manures and inorganic fertilizer on productivity of the crop and on soil fertility after rice cropping system in low land.

Due to the escalating cost of chemical fertilizer and objective of minimizing the environmental pollution the search for alternative sources of plant nutrient is imperative. The diversification of organic sources of plant nutrients is becoming popular these days and use of compost or vermicompost has become an important input in the integrated use of plant nutrient supplied. In agricultural fields of recycling of crops residues makes an appreciable contribution in improving the organic matter content of the soil. The residues influence physico-chemical and biological properties of the soil and play an important role in energy flow and nutrient cycling. If the decomposition of residues is done by composting i.e. by the use of enrichment materials or by vermiculture inoculation the decomposition becomes further accelerated and the decomposed product becomes enriched with mineralizable plant nutrient. Thus, applications of vermicompost improve soil health and sustain the production of crop. Keeping these points in view an effort was made to find out influence of vermicompost alone and its combination with chemical fertilizers on the yield and yield attributing parameters of rice.

MATERIALS AND METHODS

Field experiment was conducted to study the impact of different combination of organic sources and chemical fertilizer on rice crop in southern transitional zone of Karnataka at Kuvempu University, Shankarghatta, Shimoga during summer season 2007.

The treatments comprised of no fertilizer application, Recommended Dose of Fertilizer, FYM and different doses of vermicompost. Totally there were 7 treatments and experiment was laid out in randomized complete block design with each treatment replicated thrice. Vermicompost and well-decomposed farmyard manure were applied in the rice field as per treatment imposed during final land preparation. While applying NPK fertilizer to the test crop 50%

of nitrogen and full dose of phosphorus and potassium were applied as basal before transplanting. The rest of the quantity of nitrogen was applied in two splits as top dress, i.e., one at the active tillering stage and the other at the panicle initiation stage. Twenty-five days old seedlings were transplanted on 5th of February 2007 with a spacing of 20 cm x 10cm and harvested on 5th of June 2007. The observations on growth attributes were recorded at 30, 60, 90, and 120 days after transplanting and yield attributes were recorded form the earmarked area.

Treat mental details

T1- control

T2- Vermicompost 5 t ha-1

T3- Vermicompost 10 t ha-1

T4- Vermicompost 15 t ha-1

T5- farm yard manure 10 t ha-1

T6- Recommended dose of fertilizer at 125:62.5:62.5 kg ha-1

T7- Vermicompost 10 t ha-1 + Recommended dose of fertilizer

The data collected on different characters during the course of investigation were subjected to Fishers method of analysis of variance technique for interpretation of the data as given by Panse and sukhatme (1967). The level of significance used in "f" and "t" test was p=0.05. Critical difference (CD) values were calculated for the p=0.05 probability level wherever "f" test was found significant.

RESULTS AND DISCUSSION

Significantly varied growth components in rice were observed due to different levels of vermicompost and combined application of chemical fertilizer and vermicompost (Table 1).The integration of vermicompost at10t/ ha + Recommended NPK (Recommended Dose of Fertilizer) recorded significantly taller plants at 30, 60, 90 and at harvest (30cm, 72 cm,101.2 cm and 108 cm, respectively) compare to all the treatments except application of vermicompost at15t/ha solely. Similar trend was also noticed due to the above treatments with respective number of tillers/plant, number of leaves/plant. The increased plant height, number of leaves and tillers due to the combined application of organics and in organics might be due to faster/ more uptake of nutrient and availability of plant nutrient throughout the life cycle of the plant growth period as well as improvement in the nutrient and water holding capacity of the soil. These results are in agreement with the findings of Murali and shetty, Kale and Zhoa-Shi-Wei and Huong Fuzhen.

Yield parameter

Significant increase in the yield and yield attributing characters was noticed with the combined application of vermicompost at10t/ha + recommended NPK (125:62.5:62.5kg/ha). Application of vermicompost at15t/ha expressed the similar trend as that of combined application of vermicompost + recommended NPK (Table-2). Application of vermicompost at10t/ha + recommended NPK (Recommended Dose of Fertilizer) recorded significantly more number of panicles /plant (14.01), panicle length(26.6 cm),test weight (2.42gm) and filled grains/panicle (90.30). When compared to Recommended Dose of Fertilizer alone (12.1, 20.6, 2.30 and 84.3 respectively). But application of

vermicompost at15 t/ha recorded significantly higher number of panicles (13.6), panicle length (25.6), test weight (2.40 and filled grain/panicle (88.20) compare to rest of the treatments except in integration of vermicompostat10t/ha + Recommended dose of fertilizer treatment. Significantly increased in the grain yield was obtained with combined application of vermicompost at 10t/ha + Recommended Dose of Fertilizer (6910kg/ha) compared to rest of the treatments except in treatment that received vermicompost at15t/ha (6385kg/ha).The increase in the grain yield with combined application of vermicompost at 10t/ha+ Recommended Dose of Fertilizer over the other treatments 8.52 - 89.23%. this indicates that the rice responded to integrated nutrient management which might be due to the improved soil condition and availability of plant nutrients throughout the plant growth period .The increased yield was also due to significantly increased yield attributing parameter viz. with higher number of panicles, panicle length, test weight, and filled grains/panicles. This is in conformity with the findings of Kale and Bano and Singh. These significantly higher yield attributes and its direct influence on the yield are on also in agreement with the findings of Bhattacharjee.

The increase in grain yields with the vermicompost at15t/ha is to the tune of 61.23% over Recommended Dose of Fertilizer alone. The increased grain yield with vermicompost might be attributed due to increased availability of nutrients from vermicompost presence of beneficial micro flora such as nitrogen fixer, phosphate solubilizer VAM fungi etc and due to the presence of biologically active metabolites like Gibberellin's, cytokininis, auxins and group B vitamins.

CONCLUSION

It has been concluded that all the growth and yield parameters improved significantly due to integrated nutrient management practice (Vermicompost 10t/ha + Recommended Dose of Fertilizer) over the rest of the treatments except the treatment that received 15t/ha of vermicompost (T_4). Application of organic manures in these treatments and their subsequent decomposition in soil released the plant nutrients slowly throughout the crop growth and thus improved all the yield attributes of the crop. Application of organics to the soil will improve physical, chemical and biological properties of the soil besides sustaining the productivity of Rice.

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Plate 1: Treatment I= Control (No Fertilizer / Organic Manures application)

Plate 2: Treatment VI =Recommended Dose of Fertilizer (RDF)

Plate 3: Treatment IV= Vermicompost at 15 t/ha

Plate 4: Treatment VII = Vermicompost at10 t/ha + RDF

Table 1: .Growth parameters of rice as influenced by vermicompost and its combination with chemical fertilizers

Treatment	Plant height				Number of leaves				No. of Tillers		
	30	**60**	**90**	**120**	**30**	**60**	**90**	**120**	**30**	**60**	**90**
T_1 control	11	28	50	59	9.06	13.13	19.4	15.4	2.2	3.6	6.8
T_2- Vermicompost at 5 t ha^{-1}	23	54	74	79	15.4	43.7	67.9	63.8	3.8	11.5	13.8
T_3- Vermicompost 10 t ha^{-1}	25	60	90	93	18.4	53.8	74.6	69.8	6.2	12.46	15.1
T_4- Vermicompost 15 t ha^{-1}	30	68	96	106	20.53	69.6	86	82.3	8.8	13.47	16.06
T_5- Farm Yard Manureat10 t ha^{-1}	24	54	77	87	16.63	51.2	72.5	68.5	5.5	11.9	14.6
T_6-Recommended dose of fertilizer (RDF)	25	62	87	96	19	61.2	79.3	75.6	7.5	13	15.6
T_7- Vermicompost 10 t ha^{-1} + RDF.	30	72	101.2	108	22.13	77.3	87.8	86	9.4	15.4	18.06
SEm±	**0.43**	**1.52**	**1.76**	**2.01**	**0.67**	**2.31**	**1.43**	**1.38**	**0.34**	**0.72**	**0.83**
C.D at 5 %	**1.28**	**4.52**	**5.24**	**6.01**	**1.9**	**6.87**	**4.26**	**4.12**	**1.01**	**2.14**	**2.46**

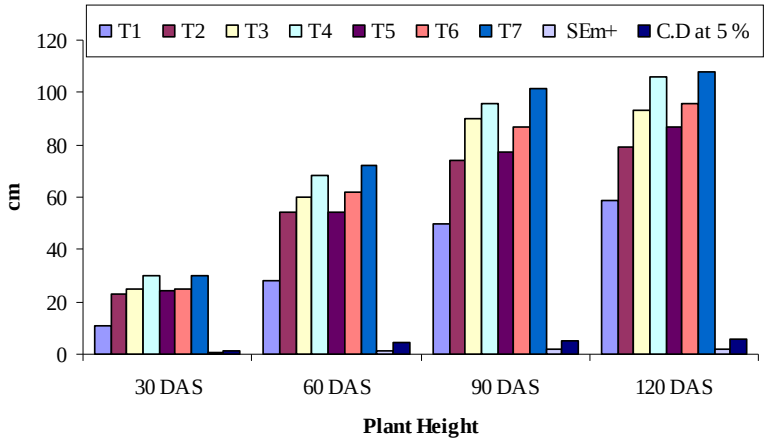
Note: RDF= Recommended dose of fertilizer at 125:62.5:62.5 kg ha^{-1}

Table 2: Yield and yield attributing characters of rice as influenced by vermicompost and its combination with chemical fertilizers

Treatment	No. of panicles	Panicle Length (cm)	Test Weight (100 grains)	Filled grains/ panicle	Grain Yield (Kg/ Ha)
T_1 control	3.5	12	2.106	22.01	744
T_2- Vermicompost at 5 t ha^{-1}	10.6	13.3	2.24	78.2	1730
T_3- Vermicompost 10 t ha^{-1}	11.2	18.3	2.27	82.3	3801
T_4- Vermicompost 15 t ha^{-1}	13.6	25.6	2.4	88.2	6385
T_5- Farm Yard Manureat10 t ha^{-1}	10.8	17	2.26	79	3446
T_6-Recommended dose of fertilizer (RDF)	12.1	20.6	2.3	84.3	3960
T_7- Vermicompost 10 t ha^{-1} + RDF.	14.01	26.6	2.42	90.3	6910
SEm+	**0.31**	**0.53**	**0.03**	**0.78**	**207.01**
C.D at 5 %	**0.92**	**1.58**	**0.08**	**2.31**	**611.64**

Note: RDF= Recommended dose of fertilizer at 125:62.5:62.5 kg ha^{-1}

A



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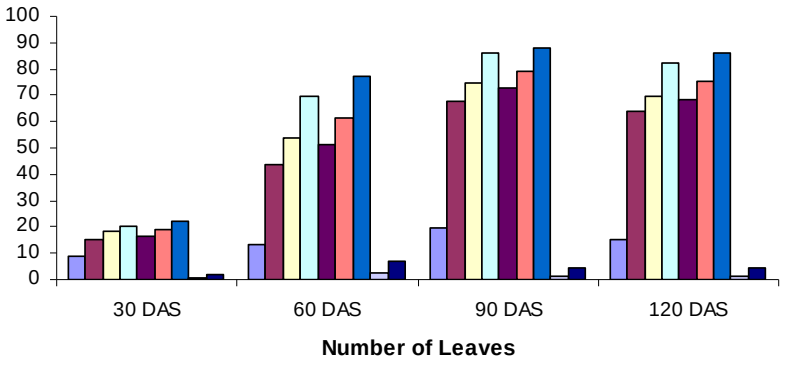

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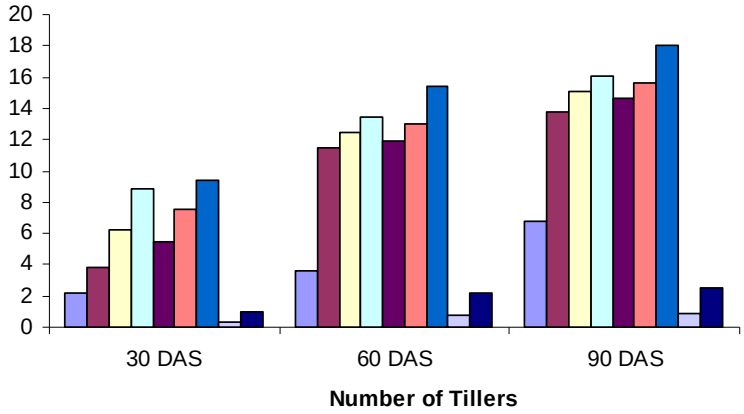
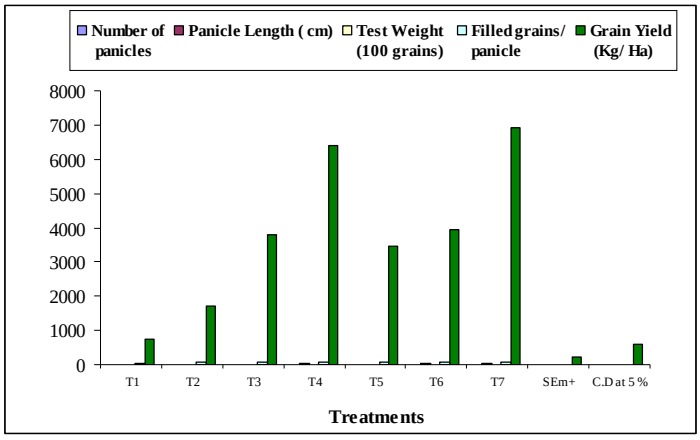


Graph -1: Growth parameters of rice as influenced by vermicompost and its Combination with chemical fertilizers



Graph-2: Yield and yield attributing characters of rice as influenced by vermicompost and its combination with chemical fertilizers

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RESEARCH ARTICLE

Pilot Scale Vermicomposting of Water Hyacinth, Pineapple Waste and Corn Bract with Earthworms Native of Tiruchirappalli,India.

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ABSTRACT

Earthworms recycle millions of tons of organic wastes, such as food waste, agricultural waste, city garbage, kitchen waste etc. Almost any organic wastes can be converted into compost within a minimum time span. The present study aims at determining the compost quality of organic waste such as Pineapple wastes, water hyacinth and corn bract using native Indian Earthworms. All the three wastes chosen had excellent potentials as a substrate for Vermicomposting.

Keywords: Vermicomposting, water hyacinth, corn bract, pineapple wastes.

INTRODUCTION

Macro invertebrates play a definite and prominent role in regulating soil processes but among them termites and earthworms play a vital role in maintaining soil fertility and in bringing about efficient nutrient cycling. Earthworms therefore, represent a key component in the biological strategies of nutrient cycling in soil and the structure of their communities gives a clear indication of the type soil system they inhabit. Earthworms recycle millions of tons of organic wastes, such as food waste, agricultural waste, city garbage, kitchen waste etc. Almost any organic wastes can be converted into compost within a minimum time span. The present study aims at determining the compost quality of various organic waste viz Pineapple, water hyacinth and corn by native Indian earthworms. The major

objectives of this study were, to study composting ability of native earthworms, compost quality of different organic wastes, Vermicomposting as resource recovery process and to make wealth out of waste.

MATERIALS AND METHODS

The earthworms were collected from Tiruchirappalli locality in areas like Holy Cross College, Ramachandran Nagar, Periyamilaguparai and Thiruverumbur areas. The worms were collected by hand sorting using spade. Around 400 earthworms were collected in all from garden soil in specific.

Substrate: Large number of vegetables, fruits and aquatic wastes are wasted at large and they possess great disposal problems. The wastes used for the present study are: *Ananas cosmosus* (Pine apple) outer skin*, Eicchornia crassipes* (Water hyacinth) collected from River Koraiyar, Trichy, *Zea mays* (corn bract).

Predigestion of substrate: Raw wastes or fresh wastes are not suitable food for worms. Hence predigestion of the raw substrates is essential for the process of composting. These wastes should be cut into tiny bits, so as to speed up the process of predigestion.1kg of cow dung and 1kg of substrate was taken in a cement tank of 60cm diameter and 70cm depth. The mixture was mixed regularly sprinkled with water to maintain moisture. Four tanks with 3 of the chosen substrates mentioned in substrates section and one tank as control with 2kg of cow dung only was kept for digestion. The sample was digested in 30days. The predigested 30days old decomposed substrate serves best food for earthworms.

Preparation of Vermibed: Cement tanks (60**×**70cm) were chosen for the study. The bed was layered with stones for 3cm to let water flow down; overlaid with 200gm of husk to prevent escape of worms followed by 3cm of sand to serve similar purpose and finally covered with the predigested substrate and 1kg of soil mixture. Earthworms were released at about 75 worms per tank and the time noted as day 0. About 250 to 500ml of water was sprinkled on the surface daily, to maintain moisture which is essential for worm growth.

Vermicompost recovery: At 45th day after introduction of worms the compost was ready for use. This was evident by its physical appearance as judged by the development of the dark brown colored loose granular mass with uniformly disintegrated structure, watering was stopped, after one or two days the compost was removed from the cement tank together with the worms, heaped on a plastic sheet and kept in the shade. The compost was removed from the top leaving the earthworms in the form of a bundle at the bottom. The compost was then sieved, dried in shade and packaged.

Chemical character of the vermicompost: The fresh castings ejected by the earthworms were collected from each of the vermibed containers and separately packed in air tight plastic bags and tested by Tamil Agricultural Department, in Soil Testing Laboratory, Tiruchirappalli, for Nitrogen, Potassium, pH and Electrical conductivity.

RESULTS

The present study is an attempt to evaluate the potency of three substrates as a resource for composting technology. The compost was studied for chemical parameters, viz Electrical Conductivity, pH, Nitrogen and Potassium .The results obtained are depicted in Table.1 and Figure.1 Highest electrical conductivity was observed in pineapple waste, 1.21dSm^{-1}. Electrical conductivity in control (with cow dung only) was higher, about 0.66dSm^{-1} and least in corn bract 0.44dSm^{-1} and water hyacinth 0.52dSm^{-1}. The pH was almost nearest to neutral range but slightly alkaline. Highest pH was observed in pine apple 7.34 and lowest pH in control 7.17 and the pH were more or less equal about

7.26 in corn bract and 7.22 in water hyacinth. Nitrogen an important parameter in composting was highest in corn bract 1.55g/100gm and least in pineapple 1.08g/100gm. The Nitrogen content of water hyacinth and control was 1.19g/100gm and 1.15g/100gm respectively. Potassium content of the substrate was tested and found to be highest in pineapple 1.75g/100gm and lowest in corn bract 0.82g/100gm. Next to pineapple water hyacinth showed higher content of potassium about 1.62g/100gm and in (cow dung) control it was 1.03g/100gm. Thus three parameters viz, Electrical conductivity, pH and potassium were highest in pineapple waste, 1.21dSm^{-1}, 7.34g/100g and 1.75g/100g respectively and the parameters - Electrical conductivity and potassium was in least quantity in corn bract 0.44dSm^{-1} and 0.82g/100gm respectively.

DISCUSSION

Exotic worms take 40 days for composting, whereas 50 days are needed for local earthworms and 80 days for conventional composting (control). In the present study the various substrate were decomposed in 45 days by native worms. Low EC, Slightly basic pH, moderate level of Nitrogen and Potassium improve agricultural yield better (Tamil Nadu Agricultural Department, Soil Analysis Lab Report). Increasing substitutions of substrate in composting caused an increase in pH, EC and macronutrients (Atiyeh,R.M, *et al* 2001). All the physicochemical parameters studied had optimal levels of the same for the various substrates. The high levels of nitrogen and potassium make these substrates applicable as organic fertilizers to the soil.

Salinity is a soil property referring to the amount of soluble salt in the soil and is indicative of the ability of an aqueous solution to carry an electric current. Electrical conductivity (EC) is the most common measure of soil salinity. Plants are affected, both physically and chemically, by excess salts in soils. By agricultural standards, soils with an EC greater than 4 dS/m are considered saline. Salinity thus plays an important role in plant growth. The EC was analyzed for the three samples and control. Electrical conductivity for 30days sample by polycultures of filter mud from sugarcane industries was between 0.14-0.25 dS/m (Khwairakpam and Bhargawa, 2009).

The electrical conductivity in the present study was very much within permissible limits 1.21 dS/m the highest for pineapple waste and least 0.44dS/m in corn bract. Electrical conductivity might result due to loss of weight by organic matter and release of different mineral salts (Garg *et al* 2006).Higher EC values were obtained in combinations of market waste and cowdung waste (1: 0.5, 1:1and1:1.5), were 3.95 dS/m, 2.60 dS/m, 2.80 dS/m for respectively.(Karthikeyan,V,*et.al.,*2007). Agro industrial waste composting by *Eudrilus eugeniae* ranged from 0. 64 dS/m - 1.32 dS/m.(Kitthurmath *et.al*.,2007).

The soil pH is important because it affects the availability of nutrients in the soil. Many plant nutrients are not readily available to plants in highly alkaline or acidic soils. These essential nutrients are most available to most plants at a pH between 6 and 7.5. pH for compost from filter mud using polycultures showed a range from 6.06-6.7 and that of monoculture from 6.16-6.06 initially(Khwairakpam and Bhargawa,2009). Goraknath and Keshavsingh,(2009), also reported that composting of different combinations of animal, agro and kitchen wastes caused a change in pH from alkaline to an acidic or neutral range 7.6 - 6.5. The shift in pH from alkaline to acidic range is attributed to the bioconversion of organic materials into organic acids (Ndegwa *et.al*.,2001). In the present experiment the pH was found to be slightly alkaline for all studied ranging between 7.17 – 7.34. A similar result with alkaline pH was obtained by Karthikeyan, et.al, 2007, wherein pH of 8.33; 8.49 and 8.3 when combinations of market waste and cowdung waste in 1: 0.5, 1:1and1:1.5 ratios respectively were used. Mainoo,NO, *et.al,*2009, showed that fresh pineapple waste had a pH of 4.4 but after 24 weeks the pH increased to about 7.2- 9.2, *ie*, an alkaline range, pineapple composting in the present study had a pH of 7.34 thus supporting our results. Chemical analysis of Vermicomposting from dry leaves of fruit trees showed a pH ranging between 6.89 & 8.60 (Sannigrahi, 2009).While alive, living things

have high nitrogen content – they are "green". When they die and start decomposing and drying out in the open, airborne bacteria and other decomposers consume the protein rich tissues first and disperse the nitrogen in the form of dead bacteria, bug droppings, ammonia and other by-products. The carbon rich plant fibers stay intact much longer. This is the process that turns green material like grass clippings that are left in the open into brown materials like dry grass. Manure is considered a green material because it stays rich in nitrogen for long. The higher degree of decomposition and mineralization in vermicompost may be one of the reasons for higher nitrogen content. Increased content of Nitrogen may also be attributed to the release of nitrogenous products of earthworm metabolism through the casts (excreta), urine as well as mucoproteins (Syres and Springett,2003; Bano *et al*.,1987). Mainoo,NO, *et.al,*2009, showed that fresh pineapple waste contained as much as 0.4% total Nitrogen, The decomposition efficiency of *Perionyx sansibaricus* on a variety of wastes such as agriculture waste, farmyard manure and urban solid waste was studied by Suthar,S, 2006 . Composting resulted in significant increase in total N (80.8-142.3%), P (33.31-114.6%) and K(26.32-125.2%), whereas decrease in organic carbon(14.0-37.0%) as well as C:N ratio(52.4:69.8%). The total Nitrogen content was found to be 1.08% - 1.55% minimal for pineapple and maximal for corn bract. *Leucaena leuccophela* Lamk, leaves were composted in two months and contained N, 0.9-1.9%; P, 0.5 – 1.2% and K, 0.9 – 2.5% while that of *Bombax ceiba* yielded 1.2%; 0.6% and 1.8% of N, P and K respectively(Sannigrahi, 2009).

High Nitrogen content were obtained in combinations of market waste and cowdung waste (1:0.5, 1:1 and 1:1.5), were about 1.78%; 1.88% and 1.82% respectively. (Karthikeyan, V, 2007). The enhanced microflora present in the gut of earthworms, during composting process plays an important role in the increase in Potassium and other nutrient (Kaviraj and Sharma 2003). Hemalatha and Meena,(2006), decomposed Municipal solid waste, vegetable waste, raw dairy distillery effluents and showed that combination with dairy effluent gave high levels of macronutrients, especially, Potassium. Suthar (2006) decomposed yard manure and urban solid waste, and reported an increase in total potassium from 26.3 to 125.2% on using *Perionyx sansibaricus* . Vermicomposting and garden compost resulted in 0.15-0.73% and 0.48% levels of Potassium, respectively (Nagavellama *et al* 2004).

Mainoo,NO, *et.al,*2009, showed that fresh pineapple waste contained as much as 0.9% total potassium. In the present experiment the total potassium was found to be highest for pineapple about 1.75% an least in corn bract 0.82%. A low Potassium content was obtained in combinations of market waste and cowdung waste (1:0.5, 1:1 and 1:1.5), which was about 1.56%; 1.62% and 1.52% respectively against an initial level of 1.85%. (Karthikeyan, V, 2007). Hence as a concluding remark vermitechnology can be viewed as an alternate resource technology and as a means to save the environment. Let us take one step towards organic farming and save Mother Earth from Pollution Hazards.

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Table 1:- Evaluation of chemical parameters in the compost materials

Chemical Substrates	Electrical Conductivity (dSm^{-1})	pH	Nitrogen g/100g	Potassium g/100g
Control	**0.66**	**7.17**	**1.15**	**1.03**
Eicchornia crassipes	**0.52**	**7.27**	**1.19**	**1.62**
Zea mays	**0.44**	**7.26**	**1.55**	**0.82**
Ananus cosmosus	**1.21**	**7.34**	**1.08**	**1.75**

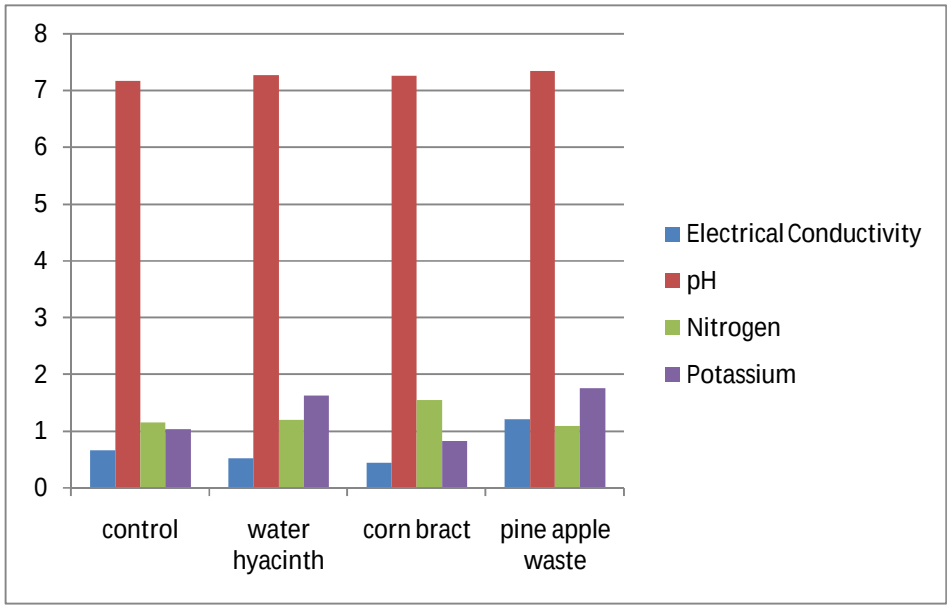

Figure 1:- Chemical analysis of different composted substrates

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RESEARCH ARTICLE

Heavy Metals Distribution in Surface Sediments of River Noyyal at Tirupur, TamilNadu, India.

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ABSTRACT

River Noyyal is one among the tributary of River Cauvery in Tamilnadu and it receives massive level of dyeing and bleaching effluents and municipal sewage while it flows through the two urbanised industrial cities namely Coimbatore and Tirupur. The present investigation attempted to assess the extent of heavy metal enrichment in surface sediments. Sediment samples were collected at three selected location in River Noyyal in urban Tiruppur during January (2008) to December, 2010) in monthly interval and analysed for physico-chemical characteristics and heavy metals. The total metal concentrations of iron, manganese, copper, chromium, zinc and lead were recorded up to the maximum levels of 4850, 545, 98, 67, 92 and 24 mg/kg, respectively. Maximum concentration of chromium (67 mg/kg) and lead was measured (24 mg/kg) at was recorded at Orathupalayam reservoir in June (2008). The organic carbon and organic matter of the sediment samples ranged from 0.62 to 3.02 % and 1.07 to 5.21%.

Keywords: River Noyyal, surface sediments, heavy metals, anthropogenic pollution.

INTRODUCTION

Heavy metals are ubiquitous in nature and gaining scientific interest due to its characteristics such as high reactivity, lithophilic nature, toxicity and non- biodegradability. These pollutants are metallic elements that have a relatively high density and are toxic even at low concentration [1]. Heavy metals are natural constituents of the freshwater environment, generally found in very low concentrations due to geogenic process including weathering and erosion. Contamination of freshwater sediments by heavy metals is a worldwide problem stemming from natural and

numerous anthropogenic activities from industrial, domestic and agricultural sector. The sources of heavy metals can be categorized into two groups such as natural sources and manmade sources. Soil weathering, volcanic eruptions and emissions, entrainment of sea salt spray and natural forest fires are significant natural sources of heavy metals [2-5]. Anthropogenic sources of heavy metals includes emissions from industries (electroplating, metal processing, tanneries, automobile, foundries, petrochemical processing, pesticide and fertilizer manufacturing industries), untreated or partially treated urban sewage, agrochemicals (pesticides and fertilizers), atmospheric deposition includes vehicular emissions and airborne suspended particulate matter are anthropogenic sources of heavy metals. Even though, many heavy metals are essential (Cu, Zn, Cr) to living organisms but some of the metals such as Hg, Pb, Sn, Ni, Se, and As are generally not required for metabolic activity and are toxic to living organisms at quite low concentrations [6]. Therefore, monitoring of heavy metals in freshwater system is highly essential to assess the safety of the environment and human health in particular.

The River Noyyal is one among the tributary of River Cauvery in Tamilnadu, originates from Velliyangiri hills on the Western Ghats and it reaches the river Cauvery about 170 km downstream. The river geographically located between 10°56' N, 76°41'E and 11°19' N, 77°56'E. Since, River Noyyal is a seasonal river mainly fed by the monsoons and the water flow is moderate for a short period during the monsoon season. The basin annually receives more than 3000 mm and 600 mm of rainfall during the southwest monsoon and northeast monsoon, respectively [7]. River Noyyal receives massive quantity of industrial effluents and municipal waste water while flowing through urban areas. A well known 'industrial city' Tiruppur is located on the bank of the Noyyal River. In this city, about 9000 knitting, processing and manufacturing units are functioning at present [8]. Most of published reports mainly concentrated on groundwater and surface water contamination in this region. There is no systematic study on surface sediment characteristics in River Noyyal was undertaken. In this background, the present study is focused to assess the status of heavy metal distribution in surface sediments of River Noyyal in Tiruppur region.

MATERIALS AND METHODS

Surface water sediments (top 15cm) were collected in polythene bags from River Noyyal in three sites (Kasipalayam, Orathupalayam reservoir and Muthur barrage) during January 2008 to December 2010 at monthly intervals. Sampling site characteristics are as below,

1. Kasipalayam – The sampling site receives industrial effluents and municipal sewages from Tirupur city.
2. Orathupalayam reservoir – The reservoir is located about 20 km downstream of Tirupur, on the border between Kangayam and Perundurai Taluks. It receives huge amount of industrial effluents and municipal sewages from upstream industries and urban settlements.
3. Muthur barrage **–** The sampling site is located about 15 km downstream of Orathupalayam reservoir and ahead of mixing point of River Noyyal and River Cauvery.

The field collected sediment samples were air dried at room temperature. The air-dried samples were homogenized using an agate mortar and pestle and sieved using a 200 micron mesh sieve. The particles with size less than 200 micron are retained in pre-cleaned plastic bottles for further laboratory analysis. The portion of the sample meant for chemical characterization was analyzed for the following parameters viz., pH, electrical conductivity, total dissolved solids using water analyzer (Model : 371, Make: Systronics) in a sediment water suspension in the ratio of 1:5. Total Organic Carbon (TOC) was estimated following Walkley and Black method. The TOC values were converted to the Total Organic Matter (TOM) by multiplying with 1.724 assuming that TOM contains 58% TOC.

Exactly 2g soil was weighed using a monopan digital balance and transferred to 500ml round bottom flask. To this a little amount of distilled water was added and swirled gently to make slurry. Then to this a mixture of each 20ml

nitric acid, 4ml Perchloric acid and 2ml Sulphuric acid was added respectively. The sample was then digested (Guna-Soxhlet extraction mantle) for 3 hours at 90°C. Appearance of brown fumes during digestion was followed by the addition of 5ml of 30% Hydrogen Peroxide. Bumping was controlled by putting glass beads in the sample getting digested. Ensured digestion was followed by the addition of little amount of distilled water to the unit. The digested samples were then cooled and filtered in 50ml standard flask. The final volume 50ml was made with distilled water. The samples were then transferred to pre-treated, laboratory cleaned, plastic vials and stored at 4°C till further analysis. The digested samples were aspirated in the Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst 800) and absorbance was noted from which estimations were made for each metal concentration in mg/kg.

RESULTS AND DISCUSSION

Monthly variation of physico-chemical characteristics of surface sediment samples of River Noyyal at three locations (Kasipalayam, Orathupalyam and Muthur barrage) for a period of three years (January 2008 to December 2010) are presented in the table. The descriptive statistics of surface sediment characteristics are presented in the tables 1. The pH of the sediment samples ranges between 7.39 and 8.92 with an average of 8.15. Maximum level (8.92) of pH was observed at Kasipalayam in the month of March (2010) followed by Muthur barrage (8.84). The pH of the sediment samples implies neutral to alkaline nature of sediments in all three sampling sites. The electrical conductivity and total dissolved solids ranged from 0.34 to 2.57 mS/cm and 209 to 1579 ppm, respectively. Maximum level (2.57 mS/cm) of electrical conductivity was observed at Muthur barrage in the month of May (2010) and minimum level measured (0.34 mS/cm) during October (2008). Values for electrical conductivity and total dissolved solids were high in almost all locations in April to July while compare to rest of the months. Significant higher level of electrical conductivity and total dissolved solids noted in summer period probably attributed to high evaporation rate and condensation of ionic content in the surface water. Analysis of variance yielded significant difference between sampling stations and sampling events (*P>0.05*).

The soil organic carbon is a common constituent of all organic matters. The organic carbon and organic matter of the sediment samples ranged from 0.62 to 3.02 % and 1.07 to 5.21%. Maximum level (3.02%) was observed at Kasipalayam in the month of August (2009) and minimum level measured (0.62%) during November (2010). In all three sampling sites, notable decline of organic matter was observed during monsoonal months where as maximum level of organic matter was observed in the months from March to June throughout the study period. Higher organic carbon in the dry months (May to June) attributed to higher rate of decomposition and mineralization of organic matter deposited at the sediment. The association of organic carbon in sediment is crucially dependent on the grain size, whereby higher organic content represents in the finer particles [9]. Thus, low organic carbon level in the study area may be because of the sediment texture which was dominated by sand (nearly 90%). Level of organic matter was observed in the order: Orathupalayam reservoir > Kasipalayam = Muthur barrage. There was a significant difference in organic matter level between stations as well as years studied (*P>0.05*).

Heavy metals are natural constituents of the freshwater environment, generally found in very low concentrations. Human activity has inevitably increased the levels of metal ions in many of this natural water system. Mining, industrial (pesticides, paints, leather, textile, fertilizers, and pharmaceuticals) and domestic effluents, agricultural runoff etc. have all contributed to the increased metal load in these waters being ultimately incorporated into aquatic sediments [10]. In River Noyyal, the relative abundance of heavy metals in sediments was measured in the order Fe > Mn > Cu > Zn > Cr > Pb (Table 1). Iron (Fe) is the most abundant metal in all sediments because it is one of the most common elements in the earth's crust. Concentration of Fe was found in the range from 2644 to 4450 mg/kg, 2644 to 4850 mg/kg and 2308 to 4645 mg/kg in Kasipalayam, Orathupalayam reservoir and Muthur barrage, respectively. Maximum level of Fe (4850 mg/kg) was recorded at Orathupalayam reservoir in June (2008) and minimum level (2308 mg/kg) was observed at Muthur barrage in August (2009). Mean maximum of Fe level (3715 mg/kg) was

observed in Orathupalayam reservoir followed by Kasipalayam (3475 mg/kg) and Muthur barrage (3405 mg/kg). Mean concentration of Fe in Noyyal river sediment samples (3532 mg/kg) were well below than the mean abundance of Fe in the earth's crust (47,000 mg/kg). Insignificant difference in Fe level between sampling events stations as well as sampling stations (*P>0.05*) suggests geogenic origin of Fe. Other Indian rivers outside of this study area had higher values in the range 5051 - 239,000 mg/kg [11-12], further suggesting that the current samples are considered to be unpolluted by Fe. Manganese (Mn) level was observed in the range from 79 to 545 mg/kg with an average of 261 mg/kg. Maximum level was recorded at Muthur barrage (545 mg/kg) in November (2008) and minimum level was measured at Kasipalayam (79 mg/kg) in June (2009). Mean concentration of Mn was observed as 184, 310 and 290 mg/kg in Kasipalayam, Orathupalayam reservoir and Muthur barrage, respectively. The mean value of Mn in River Noyyal (290 mg/kg) is much lowered compared to the average value (605 mg/kg) for Indian rivers [13]. There was a significant difference in Mn level between stations as well as sampling stations (*P<0.05*). It suggests notable input from agrochemical runoff from the upper portion of river basin where agriculture is a prime activity. Apart from the natural mineralogical sources, the only predominate source of Mn is macronutrient fertilizers application [14].

Concentration of Copper (Cu) was recorded in the range from 12 to 98 mg/kg in the study period. Maximum level of Cu was found at Orathupalayam reservoir (98 mg/kg) in the month of May (2008) and minimum level (12 mg/kg) was found in November (2009). Mean maximum of Cu content in sediments was observed at Orathupalayam reservoir (51 mg/kg) followed by Muthur barrage (48 mg/kg) and Kasipalayam (34 mg/kg). Mean Cu in sediments of River Noyyal is comparatively higher than the mean concentration of Cu (28 mg/kg) in River sediments of India [13]. There was a significant difference in Cu level between sampling stations as well as years studied (*P<0.05*).

Chromium (Cr) is considered one of the 14 most noxious heavy metals. Hexavalent form of Cr is known to cause wide range of human health effects including mutagenic and carcinogenic risks [15]. In sediments of River Noyyal, Cr level was recorded in the range from 1 to 67 mg/kg with an average of 19.4 mg/kg. Maximum concentration of Cr (67 mg/kg) was recorded at Orathupalayam reservoir in June (2008). Maximum level of Cr was observed in the summer season (April to June) throughout the study period. Concentration of Cr was observed in the order of Muthur barrage > Orathupalayam reservoir > Kasipalayam. Cr level in sediment samples recorded upto 67 mg/kg suggests contribution from industrial effluents, municipal sewage and agrochemicals in the river basin which is characterised very low water flow in most of the months (except monsoon) which facilitates accumulation on bed sediments. Earlier studies conducted in various Indian rivers observed in the range from 115 to 817 mg/kg [16]. The values obtained in this study belong to the category of unpolluted with respect to Cr while comparing with observations in the polluted sediments.

Concentration of zinc (Zn) varied between 6 and 92 mg/kg with an average of 42.5 mg/kg. Maximum level (92 mg/kg) of zinc was recorded at Kasipalayam in the month of September (2009) followed by the same sampling site (72 mg/kg) in the month of October (2009). Mean level of Zn distribution was observed in the order of Orathupalayam reservoir > Kasipalayam > Muthur barrage. Zinc (Zn) level in sediment samples were recorded upto 92 mg/kg with a mean level of 42 mg/kg. Major sources of Zn are industrial emissions, composted materials and agrochemicals (Romic and Romic 2003). Level of Zn in the sediments of Hindon River recorded in the range from 3.98 to 85.0 mg/kg [17]. However, Olivares-Rieumont et al. [18] reported in the range of 86.1 to 708.8 mg/kg of Zn in the sediments of Almendares River, Cuba. Elevated Zn exposure may leads to nausea, abdominal cramps, low copper status, altered iron function and reduced immune function [19].

Lead (Pb) is second among the top 20 priority list of hazardous substances [20]. Pb content in sediment samples figured between 3.8 and 23.6 mg/kg with a mean level of 10.8 mg/kg. Maximum level was found at Orathupalayam reservoir (24 mg/kg) in the month of April (2008) followed by Muthur barrage (21 mg/kg) was found in April (2010). Mean concentration of Pb was observed as 10, 12 and 10 mg/kg in Kasipalayam, Orathupalayam reservoir and

Muthur barrage, respectively. Pb concentration in all three sampling sites is comparatively low during monsoonal months (August to December). Significant difference was found in Pb levels in sediment samples between sampling stations as well as years studied ($P<0.05$) in River Noyyal. Earlier studies also highlighted large fluctuations in the Pb level in freshwater sediments based upon the deposition and accumulation of atmospheric particulates from anthropogenic sources. Apart from leaded gasoline, sewage sludge and agricultural runoff are the major sources of Pb contamination in aquatic environment [21]. Singh et al. [12] reported 75.30 mg/kg as a maximum concentration of Pb in the sediments of Gomti River, India. High accumulation of Pb may hinder the functions of essential mineral element (Ca, Fe, Cu and Zn) and inhibit red blood cell - enzyme systems [22].

Significant variation noted in heavy metal concentration in samples probably attributed to the grain size variation, difference in sediment components responsible for sorption and varying amounts of anthropogenic contributions [23]. The Correlation matrix of the heavy metals demonstrates good inter-relationship between Cr, Pb and Zn, and suggests a common sink of these metals into the sediments, probably resulting from industrial waste discharge and municipal sewage discharge into the river system. The present observation is similar to other polluted rivers and contaminated sediments elsewhere. Untreated or partially treated industrial effluents, municipal sewage, solid waste dumping and emissions from automobiles are prime suspected sources of metal pollution in the study area.

CONCLUSION

Heavy metals distribution in surface sediments of River Noyyal observed in the order Fe > Mn > Cu > Zn > Cr > Pb. Significant variation noted in heavy metal concentration in sediment samples probably attributed to the grain size variation, difference in sediment components responsible for sorption and varying amounts of anthrogenic contributions. Supportively, correlation analysis also demonstrated good inter-relationship between Cr, Pb and Zn, and suggests common sources of these metals. Dyeing and bleaching industrial effluents, municipal sewage discharge and atmospheric deposition are suspected sources of metal pollution.

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Table 1. Surface sediments characteristics and heavy metals distribution in River Noyyal

Parameters	Unit	Kasipalayam			Orathupalayam reservoir			Muthur barrage		
		Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
pH	-	7.4	8.9	8.0	7.6	8.9	8.1	7.7	8.9	8.2
EC	mS/cm	0.3	1.4	0.9	0.3	2.4	1.5	0.4	0.9	0.7
TDS	mg/kg	208.8	865.7	533.2	208.8	1485.9	931.0	208.8	865.7	533.2
TOC	%	0.7	2.2	1.5	0.7	3.0	1.9	0.6	2.6	1.6
OM	%	1.1	3.8	2.5	1.1	5.2	3.2	1.1	3.8	2.5
Fe	mg/kg	2643.9	4450.0	3474.8	2643.9	4850.0	3714.9	2308.0	4645.0	3404.9
Mn	mg/kg	79.0	315.0	184.1	96.0	514.0	309.6	82.5	545.0	289.7
Cu	mg/kg	12.0	67.0	34.2	21.0	98.0	51.0	20.1	74.0	47.7
Cr	mg/kg	2.5	36.0	13.8	1.0	67.0	18.4	9.0	58.0	26.1
Zn	mg/kg	6.0	67.2	40.3	37.4	92.0	57.3	12.6	64.0	29.4
Pb	mg/kg	3.8	18.8	10.5	4.2	23.6	11.5	4.8	21.1	10.2

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   List the first six contributors followed by *et al*.
2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.
3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.

Books and other Monographs

4. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
5. Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.
6. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

INSTRUCTION TO AUTHOR

Illustrations: Tables - Should be typed on separate sheets of paper and should not preferably contain any molecular structures. Only MS word table format should be used for preparing tables. Tables should show lines separating columns but not those separating rows except for the top row that shows column captions. Tables should be numbered consecutively in Arabic numerals and bear a brief title in capital letters normal face. Units of measurement should be abbreviated and placed below the column headings. Column headings or captions shall be in bold face. It is essential that all tables have legends, which explain the contents of the table. Tables should not be very large that they run more than one A4 sized page. Tables should not be prepared in the landscape format, i. e. tables that are prepared width wise on the paper.

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Chemical terminology - The chemical nomenclature used must be in accordance with that used in the Chemical Abstracts.

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Enzyme nomenclature - The trivial names recommended by the IUPAC-IUB Commission should be used. When the enzyme is the main subject of a paper, its code number and systematic name should be stated at its first citation in the paper.

Spelling - These should be as in the Concise Oxford Dictionary of Current English.

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The journal publishes exciting findings, preliminary data or studies that did not yield enough information to make a full paper as short communications. These have the same format requirements as full papers but are only up to 15 pages in length in total. Short Communications should not have subtitles such as Introduction, Materials and Methods, Results and Discussion - all these have to be merged into the running text. Short Communications preferably should have only 3-4 illustrations.

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