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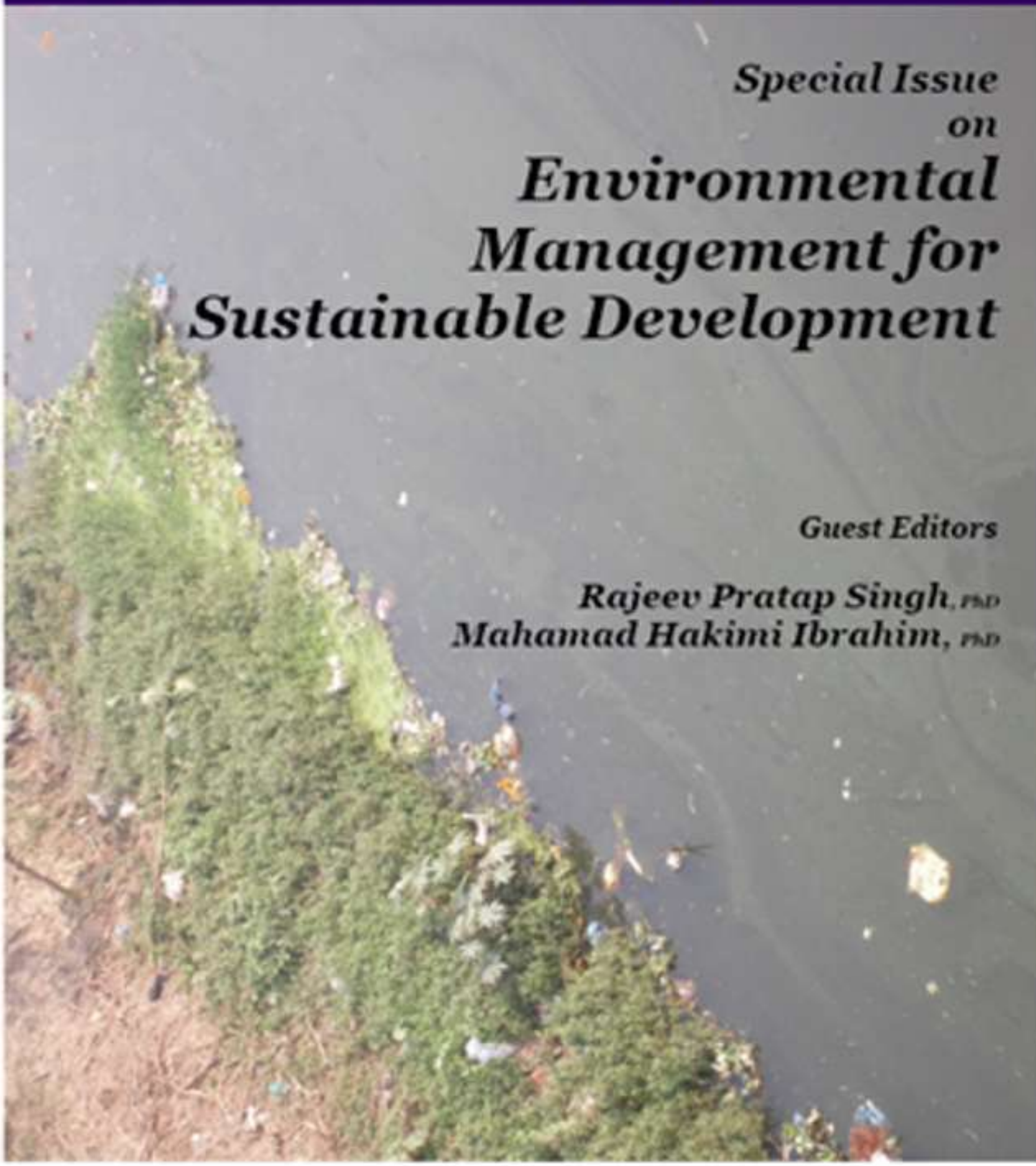

Special Issue on

Environmental Management for Sustainable Development

Guest Editors

Rajeev Pratap Singh, PhD

Mahamad Hakimi Ibrahim, PhD

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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

***Vasco Azevedo**, Editor-in-Chief*
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RESEARCH ARTICLE

ASSESSMENT OF WASTE TREATMENT AND ENERGY RECOVERY FROM DAIRY INDUSTRIAL WASTE BY ANAEROBIC DIGESTION

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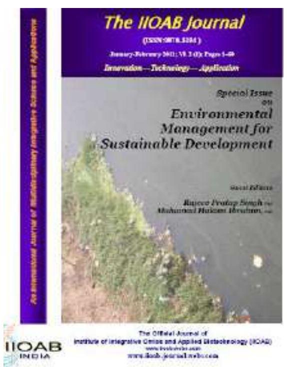
ABSTRACT



Waste treatment with simultaneous energy generation was studied in anaerobic digester using dairy industry waste (sludge, influent) as substrate. No pretreatment or solid liquid separation was applied. Batch fermentation experiments were performed with three different substrates at organic pollution load (OPL) under mesophilic range of temperature (30±2°C). Experimental data evidence the effectiveness of waste on both the removal efficiency in terms of substrate degradation and biogas yield, particularly at higher loading rates. Among the three substrates evaluated, alternative substrates showed comparatively effective performance in comparison to conventional one. However, COD removal efficiency was also found to be effective in operated environment. The described process provides the dual benefit of waste treatment with simultaneous green energy generation in the form of biogas utilizing it as substrate.

Keywords: ***waste treatment; anaerobic digestion; organic pollution load; energy recovery***

[I] INTRODUCTION

The generation and disposal of large quantities of organic waste without adequate treatment results in significant environmental pollution. Some of the waste streams are treated by conventional means like aeration, which is both energy intensive and expensive, and generates a significant quantity of biological sludge, which must then be disposed off. This biological sludge is a big problem at land-filling sites. In this context, anaerobic digestion process is increasingly recognized as economical and important step for biodegradable organic matter removal from wastewater. It is the more stable process for medium and high strength organic effluents. Apart from treating the wastewater, the biogas produce from the anaerobic process can be recovered. The anaerobic process may be perceived as a potential alternative as it not only provides renewable source of energy but also utilizes recycling potential of degradable organic portion of waste generated by a numerous activities in the country.

Anaerobic digestion is a well established process for treating many types of organic wastes, both solid and liquid [1–7, 24]. This alternative allows the recovery of energy and a solid product that can be used as an amendment of soils [8, 9]. This nutrient content of the anaerobic compost is favourable and the content of pollutants is low [10–12]. The conditions which are most important and should be considered while designing an anaerobic digestion system include-pH, temperature, total solid content, retention time, organic loading rate, carbon to nitrogen ratio and mixing.

This waste management technology capable of maintaining both environmental and energy concerns because it has dual benefits i.e. pollution control and energy production with microbiological degradation of organic pollutants, reduction of global warming potential, diminishment of odor and of course the meeting of world energy and economic needs by reducing the reliance on fossil fuels. With increasing use of anaerobic technology for treating various process streams, it is expected that industries would become more economically competitive because of their more judicious use of natural resources. Therefore, anaerobic digestion technology is almost certainly assured of increased usage in the future. Anaerobic treatment converts over half of the effluent COD into biogas [13]. Anaerobic treatment can be successfully operated at high organic loading rates; also, the biogas thus generated can be utilized for steam generation in the boilers thereby meeting the energy demands of the unit [14]. Further, low nutrient requirements and stabilized sludge production are other associated benefits [15].

Now a day water resources are polluted by varied sources, the most critical of which are city sewage and industrial waste discharge. Sewage contributes about 60 % of the total pollution load in terms of biochemical oxygen demand (BOD). In the industrial sector, water pollution is caused by a few industrial sub sectors (food processing industries, paper and pulp industries, textile, agro based industries and chemical industries), which release toxic wastes and organic pollutants [16]. Among all these industrial sub sectors, food-processing industries (dairy, edible oil and confectionary) are the major contributor for wastewater generation. The wastewater from food-processing industries is very rich in organic contents and may be a potential source for production of methane gas. There are over 18,550 food processing industries in India, emanating large quantities of wastes [17]. These wastes are either uneconomically utilized or disposed off without treatment, thereby causing serious pollution problems. With the 50% of moisture content or above, it is found that bio-conversion processes are more suitable than thermo-conversion process [18]. Wastewater from a dairy operation consists of water that has been used for plant washing, processing and cooling purposes [19]. Water management in the dairy industry is well documented [20], but effluent production and disposal remain a problematic issue for the dairy industry. To enable the dairy industry to contribute to water conservation, an efficient and cost-effective effluent treatment technology has to be developed. To this effect, anaerobic digestion offers a unique treatment option to the dairy industry. Not only does anaerobic digestion reduce the COD of an effluent, but little microbial biomass is produced. The biggest advantage is energy recovery in the form of methane and up to 95% of the organic matter in a waste stream can be converted into biogas [21].

Therefore, the objective of this work was to assess anaerobic digestion of dairy industrial waste (sludge and influent). To these purpose seven anaerobic digesters with and without cattle dung were operated in two phases. All digesters were fed with various mixing concentrations to determine the removal of organic load and energy generation from the system.

[II] MATERIALS AND METHODS

2.1. Waste collection and storage

The wastewater (influent) and sludge were collected from Dairy Industry Pvt. Ltd. Lucknow, whereas cattle dung was collected from the nearby area of the university campus of BBAUniversity, Lucknow (India). Collected sample were stored in plastic container at 4°C prior to use.

2.2. Experimental set-up

Total experimental study was divided into two phases. In Phase-I set-up all the three digesters contain pure feedstock materials (P1, P2 & P3) whereas Phase-II has mix compositions of pure feedstock materials (M1, M2, M3 & M4) with inoculum, are in the anaerobic condition with suitable temperature, required for anaerobic process depicted in **[Table–1]**.

Table: 1. Composition of waste slurry digesters and control digesters

Serial No.	Raw Material	Composition
	Phase-I	
P1	Sludge +Distilled water	1:1 = 2.5 liter
P2	Influent (pure)	1:1 = 2.5 liter
P3	Cattle dung+Distilled water	1:1 = 2.5 liter
	Phase-II	
M1	Sludge+cattle dung	1:1 = 2.5 liter
M2	Sludge +Influent	1:1 = 2.5 liter
M3	Sludge +Influent+Cattle dung	1:1:0.5 = 2.5 liter
M4	Influent (pure) +Cattle dung	1:1:0.5 = 2.5 liter

Batch experiments were carried out in two phases with identical digesters of 5 L capacity each with liquid displacement system for biogas collection. The containers were made air tight with a rubber stopper through which a gas collection tube passed. The other end of the tube was connected to a bottle, which was filled with alkali solution (2N KOH) to dissolve the amounts of CO_2 and H_2S gases.

The digester containers were maintained at mesophilic temperature 30±2°C with temperature controlled water baths. The stirring of water in the tank to obtain a uniform temperature throughout, was done by circulating water with the help of motor pump. The digesters were fabricated using leak proof sealing along with proper inlet and outlet arrangements.

Digester feed was prepared using without and with inoculum mixing it with dairy industry waste samples for both phases. In the phase-II study, use of cattle dung as inoculum is the main feature with sludge and wastewater at various concentrations. Inoculum is one of the factor which have a wider role in energy (biogas) production. The feed was homogenized in mixing tank with the help of manual mixing mechanism. The required volume of homogenized slurry was then fed into the experimental digester. Feeding was done once in all the digesters.

Total experiment was carried out for 50 days. The content, of the digesters were mixed thrice a day by stiring manually upto 10 minutes to maintain intimate contact between the micro-organism and substrate. Details of reactor operation are given in Table– 2.

Table: 2. Details of digester operation

Mode of digester operation	Periodic conditions
Digester microenvironment	Anaerobic
HRT	50 days
Operating Temperature	30±2°C
Feed Volume	2.5 liter
Digester Volume	5.0 liter

2.3. Analytical methods

Chemical Oxygen Demand (COD), Bio-chemical Oxygen Demand (BOD), Total Solid (TS), Total Organic Carbon (TOC) and pH analysis were performed at the Environmental Quality Lab as described in Standard Methods of APHA, 1995 at pre and post digestion period for both phases. Carbon, measured as TOC (total organic carbon), is a main factor for the energy content of organic compounds. Therefore the carbon balance very much represents the energy balance in sewage/sludge treatment processes. Gas production was monitored every fifth day by a water displacement device attached to each digester.

[III] RESULTS AND DISCUSSION

Analysis of pre & post digestion period i.e. on Day 1 and after 50 Days HRT for initial and final slurries from all the digesters is presented in Table 3. Percentage removal of organic loading in context of TS, BOD and COD for waste treatment is also given in **Table–3**.

3.1. Phase-I

Two replicate digesters of pure feedstock's containing the same amount of anaerobic feed material were run to determine the reduction in OPL, TS and pH variation simultaneously for biogas generation.

3.1.1. Organic pollution load removal (COD, BOD & TOC) with TS and pH variation

When the pH values of the final slurries from first phase digesters are considered **[Table–3]**, it was observed that slightly increase in the range of digesters i.e. 8.7 (P1), 6.2 (P2), & 8.0 (P3). During anaerobic fermentation, micro-organisms require a neutral or mildly alkaline environment for efficient gas production. According to literature [22–24], a pH between 7 and 8.5 is optimum range for increased gas yield. When the corresponding COD and BOD data are considered, it is observed that initial compositions had a very high organic content. Reduction in organic pollution load is measured as total COD and BOD removed from the digesters after 50 days HRT. These values (1040mg/l (P1), 1413mg/l (P2) & 1280 mg/l (P3) for COD and 336 mg/l (P1), 356 mg/l (P2), 682 mg/l (P3) for BOD) were measured on Day 1 when the digesters have been started with pH values 8.3 (P1), 5.5 (P2) and 7.2 (P3). But, when the final slurries were observed for COD and BOD data, remarkable decreases in the values (885 mg/l (P1), 1260 mg/l (P2) & 520 mg/l (P3) for COD and 201 mg/l (P1), 200 mg/l (P2) & 500 mg/l (P3) for BOD) were measured on Day 50. At the same time, the organic input is decreased by about 11-60% (in terms of COD). Highest % reduction (59.3%) in organic load was measured with P3 digester only, which consists of pure cattle dung as slurry **[Figure–1]**. TOC values are also observed, at initial and final day of the experiment, a gradual decrease in values has been measured. These results fully support the experimental data i.e. the lowest conversion of Phase-I study with pure feedstock slurries to biogas was not obtained at P1 & P2 digesters due to very less amount in % removal (9.6 to 32.8 %) in **Figure–2**.

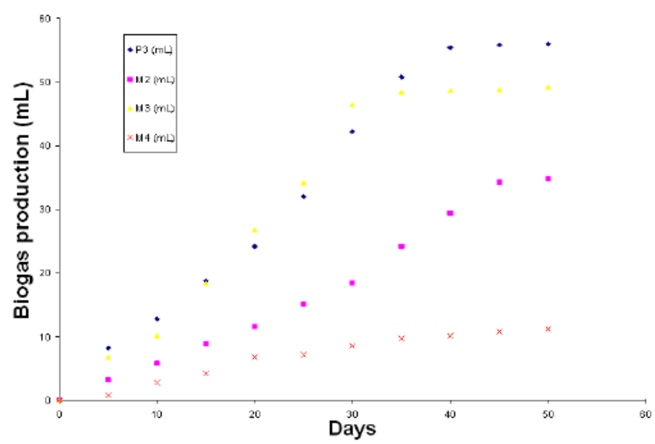


Fig: 1. Cumulated biogas production at 50 days HRT

Generally, the purity of water is determined by the amount of organic matter that it contains in terms of BOD & COD. Similarly, biodegradable potential of the material can be easily accessed by the determination of BOD and its comparison with COD. BOD determines the amount of oxygen required for microbial decomposition in a five days test at 20°C while COD indicates the amount of oxygen necessary for chemical oxidation. According to literature, if BOD/COD ratio is more than 0.6, the organic matter is easily biodegradable if it is between 0.3 to 0.6, then it points to the possibility of biodegradation, and when the ratio is 0.3, then it is not bioamenable [25].

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This statement clearly support the results of experimental Phase-I because minimum biodegradation shown by P2 (0.22) where as maximum rate recorded in digester P3 (0.53).

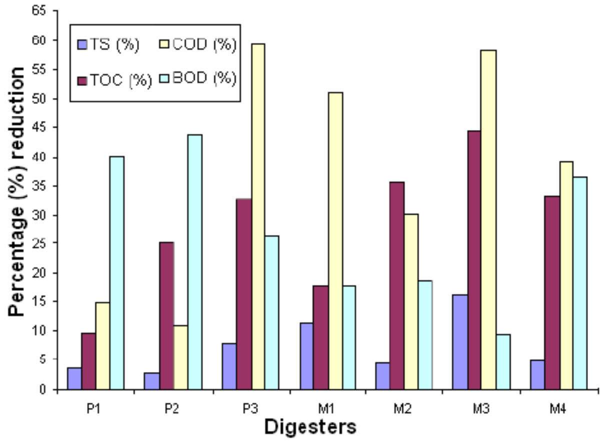

Fig: 2. Percentage (%) reduction in organic pollution load of Phase I & II digesters during study

However, in the present study maximum % reduction was observed for TS with digester P3 (7.8 %) and minimum with digester P2 (2.8 %)

3.1.2. Biogas generation

On an average the biogas production was observed every fifth day interval of total study because no rapid increase was observed for all the digesters for this phase. P1 & P2 follow the same trend i.e. no gas generation till end of the study whereas P3 followed by an increase in biogas generation rate between Days 5-35. Starting with Day 10 biogas production increased gradually up to 55.4 ml/day (Day 40) and then progressively stagnant. A possible explanation is that the soluble and/or easily degradable part of the organics contained in the P3 digester was digested

Table: 3. Pre and post digestion analysis of slurries at 50 days HRT for Phase I & II

Digesters	pH		TS (mg/l)			TOC (mg/l)			COD (mg/l)			BOD (mg/l)		
	Initial	Final	Initial	Final	% Removal	Initial	Final	% Removal	Initial	Final	% Removal	Initial	Final	% Removal
Phase I														
P1	8.3	8.7	4100	3950	3.7	1300	1175	9.6	1040	885	14.9	336	201	42.1
P2	5.5	6.2	3500	3400	2.8	700	525	25.0	1413	1260	10.8	356	200	43.8
P3	7.2	8.0	3900	3600	7.8	16000	10750	32.8	1280	520	59.3	682	500	26.6
Phase II														
M1	7.8	8.0	3200	2840	11.3	1700	1400	17.6	1962	960	51.0	705	580	17.7
M2	6.9	7.5	4500	4300	4.4	2450	1575	35.7	1236	863	30.1	250	204	18.4
M3	7.0	7.2	3700	3100	16.2	1800	1000	44.4	962	403	58.1	500	453	9.4
M4	7.0	8.0	6200	5900	4.8	4500	3000	33.3	1160	706	39.1	560	355	36.6

3.2. Phase-II

The objective of running Phase-II was two fold: to confirm the improvement in the waste treatment relative to Phase I, and to improve the biogas production observed for Phase I with addition of inoculum (cattle dung). And as a result, significant improvement was observed using inoculum with feedstock materials of Phase-I study for both parameters i.e. waste treatment through % removal in organic pollution load and energy recovery.

3.2.1. Organic pollution load removal (COD, BOD & TOC) with TS and pH variation

Because of the very high COD at initial stage (Day 1; 962-1962 mg/l), anaerobic treatment with biogas recovery is employed extensively as the first treatment step for this study. Anaerobic process reduces the organic pollution load and brings down BOD to 9.4- 40% and COD 39-58% from original initial value [Figure–2]. Moreover, anaerobic treatment is a slow process and typically requires long start-up periods but it can be reduce using inoculum as a seed material. Its participation in the metabolic reactions involving biogas generation was evident from reduction in substrate concentration by OPL (as COD, BOD, TOC) in all the experimental digesters studied. It appears from Table 3 that these compositions of feedstocks in digesters had the highest content of TS, TOC, BOD and COD at Day 1.

It is generally believed that higher degradation in total solids content result in higher bacterial growth and metabolic activities. However, in the present study maximum % reduction was observed with digester M3 (16.2 %) and minimum with digester M1 (4.4 %) for TS. When the pH values of digesters are considered, it was observed that slight increase in the values after the total operation period. However, minimum pH value (6.9) in the digester M1 on Day 1, after which pH started to increase.

According to Table 3, minimum biodegradation rate related to BOD/COD ratio shown by digester M1 (0.20) where as production for all the digesters in Phase-II are summarized in the descending order as shown by M3 > M2 > M4, whereas digester M1 showed nill production of biogas. The reason may be the absence of nutrient availability and lack of water to enhance the microbial activity. The average cumulative biogas productions observed in M2 to M4 are shown in Figure 1. Similar to Phase-I, digester P3, a rapid initial biogas production was followed by a gradually increased from Day 10 to Day 40 and its may be due to degradation of soluble or easily degradable part of the organics in feed stocks was followed by hydrolysis/solubilization [Figure–1]. The total biogas production for digester M2, M3 and M4 was found to be 34.8 ml, 49.2 ml and 11.2 ml respectively for the entire operation period. Results indicated that after addition of cattle dung with dairy waste as inoculum resulted in a much more efficient energy producer as observed in Phase-I. Among all the four digesters from Phase I & II (P3, M2, M3 & M4), it can be concluded that both dairy industry waste product (sludge and influent) shows synergistic behavior in the presence of inoculum.

3.2.2. Biogas generation

The addition of inoculum with sludge (M1), sludge & influent (M3) and influent wastewater (M4) as co-substrates facilitated effective biogas yield due to presence of readily available carbon source, whereas only sludge & influent (M2) composition without inoculum also the part of Phase-II. The order of biogas production for all the digesters in Phase-II are summarized in the descending order as shown by M3 > M2 > M4, whereas digester M1 showed nill production of biogas. The reason may be the absence of nutrient availability and lack of water to enhance the microbial activity.

The average cumulative biogas productions observed in M2 to M4 are shown in Figure 1. Similar to Phase-I, digester P3, a rapid initial biogas production was followed by a gradually increased from Day 10 to Day 40 and its may be due to degradation of soluble or easily degradable part of the organics in feedstocks was followed by hydrolysis/solubilization (Figure 1). The total biogas production for digester M2, M3 and M4 was found to be 34.8 ml, 49.2 ml and 11.2 ml respectively for the entire operation period.

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Among all the four digesters from Phase I & II (P3, M2, M3 & M4), it can be concluded that both dairy industry waste product (sludge and influent) shows synergistic behaviour in the presence of inoculum.

[V] CONCLUSIONS

The results of this study indicate that parameters of dairy industrial waste like BOD, COD, TS and TOC show a higher rate of % reduction in values after anaerobic digestion and simultaneously potential for biogas production in waste was also noticed.

According to Phase-I result, it can be conclude that pure dairy sludge and waste water was not a potential source for gas generation at individual level. However, in Phase-II, sludge, waste water and cattle dung, in combination produced potential gas production with maximum COD removal efficiency comparative to pure feedstock's of Phase-I. Similarly, high organic pollution load, absence of toxic chemicals and availability of large quantity of dairy industrial waste (sludge and waste water) may be considered as potential source for waste treatment and biogas production by anaerobic fermentation at the same time. Hence, the system is comparatively easy to operate and cost efficient in sustainable approach and the end products of anaerobic digestion are natural gas (methane) for energy production, heat produced from energy production, nutrient rich organic slurry, and other marketable inorganic products.

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RESEARCH ARTICLE

PLASTIC BAGS – THREAT TO ENVIRONMENT AND CATTLE HEALTH: A RETROSPECTIVE STUDY FROM GONDAR CITY OF ETHIOPIA

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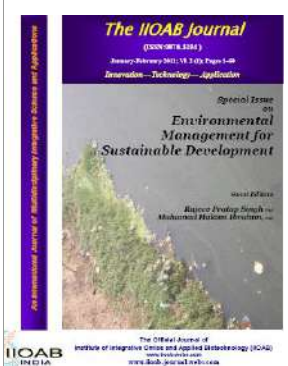
ABSTRACT



A retrospective study was conducted in Gondar city of Ethiopia for six years (2004/05 to 2009/10) to observe the impact of plastic bags usage on environment and cattle health. Paper packaging is vanishing slowly in the city and limited to small shops only. Open dumping of plastic bags containing wastes is observed commonly near road side, open plots, river side, in drains and public places however, it is prohibited under Ethiopian law. Winds carry bags to distant areas sometimes found entangled on the trees and shrubs create nuisance. During rainy season, the blockage of drains and overflowing of water was observed in some areas of the city. During study period, out of 711 rumenotomies done, in 111 (15.61%) and 600 (84.39%) animals, emergency rumenotomy and elective rumenotomy was performed, respectively. The quantity of the foreign bodies (FB's) collected from the rumen was ranging from 0.75 to 2.0 kg in 28 animals (3.94%); 2.0 to 5.0 kg in 116 animals (16.32%); 5.0 to 9.0 kg in 217 animals (30.52%) and above 9.0 kg in 350 animals (49.23%). Due to absence of plastic recycling unit in the Gondar city or in nearby areas, there is no practice of collecting and selling these products to junk dealers. Use of reusable bags made of cloths, jute and other natural fibers must be encouraged. In order to save the life of animals, residents should not pack and throw the food items in plastic bags. The cattle owners may be advised not to allow their cattle to freely wander in streets especially in the cities. They should see that the grazing lands are not polluted with the polythene and other wastes. Awareness may be created on careless disposal of plastic bags and as well as the periodical cleaning of these wastes in the grazing area.

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Keywords: *plastic bags; cattle health; environmental effects; rumenotomy; reuse; Gondar*

[I] INTRODUCTION

Every year trillions of polythene bags are used in the World. They persist on this earth to haunt us and our generations for centuries. Polythene chokes the drains and the water bodies, pollutes the land and poisons us slowly but surely. Even mowed grass cannot escape the polythene menace [1]. Polythene has been recovered from the rumen of countless cattle and is a major threat to animals also. Polythene pollution is an epidemic now. Polythene is indestructible. One particle of polythene is further made of many particles. If we continue to use polythene, the earth would become polluted on an alarming rate [2].

The word plastic has its origin from the Greek word "plastikos", which means 'able to be molded into different shapes' [3]. They are made up of long chain polymeric molecules [4] and basic materials used for their manufacturing is extracted from oil, coal and natural gas [5]. Plastic or polythene bags commonly known as festal in Amharic language are in common use as shopping bags for packaging food, and other items in Gondar city as well as other parts of Ethiopia. In addition to polythene bags, plastics

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are used in bottling of mineral water, cold drinks and liquid soap, which become part of waste after use. Majority of the residents collect their household wastes in plastic sacks and place them roadside on every Friday until it is carried away by private waste collectors in the city. Their better physical and chemical properties of being strong, light in weight, resistance to water and most water-borne microorganisms make them preferable choice over paper and reusable cloth and jute bags. Plastic consumption is growing at a rate of approximately 5% annually, and the global production reaching about 150 million tons per year [6].

After their entry to environment, plastics resist biodegradation and pollute for decades and centuries [7], and pose risk to human health and environment [8]. They are resistant to moisture, travel long distances because of their light weight, block drains during rains, and may also trap birds. Plastics cause "visible pollution" as they contribute to large volume of total municipal solid wastes and are major threat to air [9], oceans [10], soil [11], livestock [12,13], wildlife [14] and marine life [15]. Approximately, 95% of urban stray cattle in India are suffering from various ailments due to hazardous materials, mostly plastic bags inside their abdomen [13]. These plastics reduce the rain water percolation, affecting the ground water recharge and level. Soil fertility and seed germination is also affected when these plastic bags become part of manure and reaches agricultural fields. Presently there is no recycling unit of plastic bags in Gondar city so most of these plastics become part of municipal waste. As per literature survey limited work is done on this aspect in Ethiopia [16], so this study was designed to assess the impacts of plastic usage on environment and cattle health and to put forward some alternatives about plastic bags usage. The data obtained would be a baseline for the further research.

[II] MATERIALS AND METHODS

2.1. Study area

Gondar city, the capital of North Gondar zone in Amhara regional state, is located 750km North-west of capital city of Addis Ababa [Figure–1]. It is situated between 12°36′N and 33°28′E at an altitude of about 2300 m above mean sea level with an average temperature of 20°C and an average annual rainfall of 1800 mm. Being a highland area, the city is spread on different mountains, slopes and in valleys and has three small rivers, many streams and a lake. The city with a population of 186,077 [17], has 21 kebeles (wards), one hospital, three health stations, two health centers, one university, three veterinary clinics, four colleges, six secondary schools, one preparatory school (Senior Secondary School), one technical and vocational school, 27 primary schools, 13 kindergartens and an airport. The city has historical importance of being the capital of Ethiopia from 1635 to 1855 G.C. and has many medieval castles and churches.

2.2. Methodology

The retrospective study period was six years (2004/05 to 2009/10) and the data was collected by:

1. Personal observation.
2. Case study analysis i.e. by observation of cases registered under three veterinary clinics.
3. Key informant interview i.e. by conducting interviews of animal health assistants working in veterinary health service and private veterinary practitioners of Gondar city.
4. Transect walk observation.

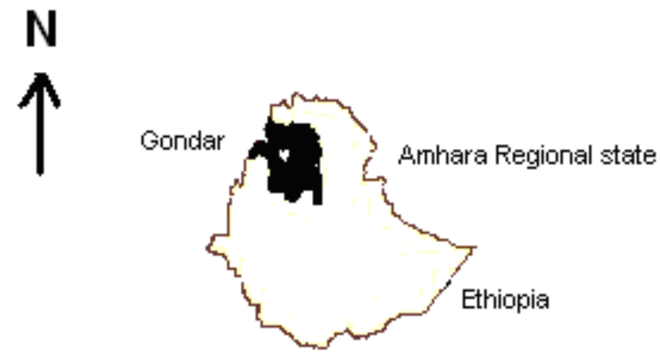

Fig: 1. Map of the study area

[III] RESULTS AND DISCUSSION

3.1. Increasing trend of plastic bags use

During the study period, authors observed an increasing trend of using plastic bags among Gondar city residents. A developing trend of "use and throw-away" towards these polythene bags is the main cause of the problem. During 2004-2008, shopkeepers sell these bags and provide the bags to those customers purchasing more items. The trend changed slowly and up to 2009, these plastic bags were given free of charge to shoppers. In some years residents of the city will be accustomed to plastic bags use that they find it difficult to change their habits. Paper packaging is vanishing slowly in the city and limited to small shops only. Locally made bags from big empty plastic sacks are in use on Saturday market day in the city but they are also losing their charm in the race of modernization. These plastic bags do not contribute much in terms of volume and weight to municipal solid waste (MSW), but the main problem is the disposal of these bags after use. Presence of used bags causes aesthetic disturbance of the city's hilly landscape.

3.2. Current practices of plastic bags use and their environmental impacts

Open dumping of residential wastes including plastic is observed commonly in almost all wards of the city. Dumping is commonly observed near road side, open plots, river side, in drains and public places however, it is prohibited under Ethiopian law [18]. Residents used commonly plastic sacks and polythene bags for storing their wastes, which ultimately become part of MSW. From the temporary and final waste disposal sites, the stray and other domestic animals like cattle and donkeys engulf these plastic bags containing food materials inside. Sometimes the street boys were also found to sort large size and good quality

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bags for their use. Winds carry away these bags to distant areas which were sometimes found entangled on the trees and shrubs and creates nuisance. During rainy season, the blockage of drains and overflowing of water was also observed in some areas of the city. Sometimes residents pack and throw the food items in the plastic bags. The stray and other animals were unable to open these bags and were found to consume whole plastic bags. In due course of time, the amount of plastic bags got accumulated in the stomach of these animals and create health problem for these animals. Intentional open burning of waste along with plastic bags is also a commonly observed practice in the city leading to problem of local air pollution with harmful gases.

As per the section 8 of Ethiopian Government Proclamation, passed in February 2007, any unlabeled plastic bag will be treated as unlawful and also bans the manufacture and import of plastic bags less than 0.03 mm in thickness [18]. Authors observed different colors of unlabelled plastic bags available and provided commonly in Gondar city, which are against the government law and need to be controlled.

3.3. Effects on cattle health

The plastic bags along with other foreign bodies in cattle affect the health and cause economic loss to the owner. The cattle have three non-glandular fore-stomach compartments - the rumen, reticulum and omasum. These are the sites for fermentative digestion. The fourth glandular compartment, the abomasum is the "true stomach" which is responsible for the next phase of enzymatic digestion. The indiscriminate eating habits and mineral deficiency make them susceptible to inadvertent ingestion of foreign materials [19, 20]. The various pathological conditions that are encountered due to ingestion of plastic and polythene materials in animals are indigestion, impaction, tympany, polybezoars, and immunosuppression [13]. The most common symptoms observed in the affected animal were bloat and were exhibited by the abnormal bulging of the paralumbar fossa on the left side of the abdominal wall. The other clinical symptoms were depression, complete or partial anorexia followed by loss of weight, ruminal impaction, reduction of milk yield, and suspended rumination. Milk and weight reduction in the affected animals was variable according to the stage of lactation, quantities of foreign bodies ingested and severity of the bloat. However accurate data in this regard was not maintained properly by the respective clinics. Acute bloat causes more pressure over the diaphragm and ribs which limits the respiratory movements, leading to hypoventilation and decreased venous return to the heart [19]. Lack of emergency and timely treatment of acute bloat may lead to cattle mortality. When the conservative line of treatment fails to correct these ailments of rumen, the only alternative is rumenotomy, which is surgically opening the rumen for treating its various ailments and to remove a variety of foreign bodies. A total of 711 rumenotomies were performed in the Gondar city area during the study period **[Table– 1]**.

The bloat or tympanites may occur in different forms like acute, chronic recurrent, simple or frothy bloat. Out of the 711 rumenotomies done, in 111 (15.61%) and 600 (84.39%) animals, emergency rumenotomy and elective rumenotomy were performed, respectively. The ruminal tympany occurs during obstruction due to foreign bodies occluding the passage of eructing gases [26]. Farmers who could not arrange timely treatment used to lose their animals. The acute bloat was due to the complete obstruction of the rumino-reticular orifice and the ingesta could not be moved to the next compartment and the stasis resulted in accumulation of the fermented gas in the rumen.

In the cases of recurrent bloat the distension of the rumen reduced subsequent to conservative line of treatment. In these cases the obstructing foreign bodies were shifted to other parts of rumen resulting in temporary relief of bloat which recurred after few days. Ruminal impaction is due to the obstruction caused to the movement of the ingesta to the next compartment and it was noticed in 148 (20.83%) animals. It has been observed that cows with polythene materials in their stomach suffer from immunosuppression that leads to increased sensitivity to various infections [13].

Table: 1. Details of rumenotomies performed in the Gondar city during 2004-2010

Sl. No.	Place of surgery	Number of cattle operated
1.	Gondar university veterinary clinic	85
2.	Gondar woreda veterinary clinic	99
3.	Animal health assistants (private practice)	502
4.	Private veterinary clinic, Gondar	25
Total		711

Table: 2. The details of quantity of the foreign bodies (FB's) collected during rumenotomy

Sl. No	Quantities of FB's recovered (Kg)	No of animals	%
1.	0.75 - 2.0	28	3.94
2.	2.0 - 5.0	116	16.31
3.	5.0 - 9.0	217	30.52
4.	> 9.0	350	49.23
Total		711	100

The foreign bodies recovered by transruminal exploration during rumenotomy are broadly classified into penetrating (nails, wires) and non- penetrating foreign bodies (polythene bags, plastic materials, rubber articles, leather pieces, and cloths) as found in the cattle **[Figure– 2 and – 3].** Other workers in different part of World encountered metallic objects [21], cloths [22] and polythene bags [23] in the gastrointestinal tract of ruminants, causing ruminal obstruction and occlusion. The foreign bodies were mostly dark black in color and their shapes modified into

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ball-like and other shapes and their texture was also altered to be tougher than original. The above changes in foreign bodies were

Fig: 2. Large quantity of foreign bodies recovered after rumenotomy.

Fig: 3. Varieties of foreign bodies removed from a cow

due to churning action by the contraction of the rumen and reticulum and also by the action of the micro flora population inside. These large tight balls inside the rumen cause impaction. In some animals the churned up masses get fused together and make it difficult to remove through the rumenotomy opening and have to be cut into pieces and removed. The quantity of the foreign bodies collected from the rumen were ranging from 0.75 to 2.0 kg in 28 animals (3.94%); 2.0 to 5.0 kg in 116 animals (16.31%); 5.0 to 9.0 kg in 217 animals (30.52%) and > 9.0 kg in 350 animals (49.23%) **[Table–2]**. The items rarely recovered were a small boy's trouser, belt and screw driver. In some unusual cases about 70% of the rumen was impacted with the foreign bodies. On exploratory rumenotomy twenty-two hair balls were recovered from the rumen and reticulum of a goat [24]. In an unusual case of rumenotomy in a cow revealed a large empty cement bag with its part embedded in the reticulo-omasal orifice obstructing the ingesta [25].

In all the cases the animals showed symptoms of pain exhibited by depression, arched back and grunting. Apart from this there was sudden drop in milk yield and 75% reduction was noticed incurring a heavy economic loss to the farmer. Losses or costs of several kinds occur like losses by death and by culling of bloat prone animals, losses of production from animals which suffer from bloat and survive, losses due to the disruption of normal farm work and management programs, losses due to the use of less productive but safer pastures, and the cost of preventive measures and treatment. Not measurable in monetary terms is the mental and physical strain on the farmer and his family when an outbreak occurs. Other costs those of research and extension services must be added [27].

3.4. Reuse and recycling

It's very difficult to impose complete ban on plastic bags use and plastic packing until there is fully equitable alternative available. The four R's as feasible options for achieving reduced material use and waste generation are Reduction, Reuse, Recycling and Recovery. Presently only reduce and reuse option are feasible in Gondar. As there are no recycling units for plastics in the Gondar city or in nearby areas, there is no practice of collecting and selling these products to junk dealers. However, the residents were found to reuse good quality of big size plastic bags. The reuse of these bags was observed among small shopkeepers and rural people, who sell vegetable in Saturday market. The residents had a good practice of reusing mineral water and cold drinks plastic bottles for pouring milk, oil, and "tella", a local beverage therefore contributing in reducing the amount of waste. In our previous study, the respondents were agreed to participate in segregating and storing the wastes provided there is some recycling unit which can buy their segregated wastes [28].

3.5. Options

Reusable bags made of cloths, jute and other natural fibers are durable, having long life span, biodegradable and above all environment friendly. Further reusable bags are washable, easy to carry and handle, will not break under the weight of heavy shopping items, reduce use of plastic bags, and do not pose a threat to environment and wildlife. Such types of bags are already in use in Addis Ababa, the capital city of Ethiopia and also seen occasionally in Gondar. Even plastic bags can be reused again and again so that threat to environment and life can be reduced. If possible we can refuse unnecessary packing of purchased item in plastics. In order to save the life of cattle, residents should not pack and throw the food items in plastic bags. The cattle owners may be advised not to allow their cattle to freely wander in streets especially in the cities. They should see that the grazing lands are not polluted with the polythene and other wastes. Awareness may be created on careless disposal of plastic bags and as well as the periodical cleaning of these wastes in the grazing area. Creating awareness among city residents regarding indiscriminate use and disposal of plastic bags will be a good option to overcome the problem in future. The non-governmental organization named "SOS Addis Tefetron Bemalimat Bikletin Masweged Mahiber" in Addis Ababa is engaged in generating awareness among Addis Ababa residents [29]; however such types of activities are needed in other parts of Ethiopia.

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RESEARCH ARTICLE

APPLICATION OF ANAEROBIC BIOTECHNOLOGY FOR PHARMACEUTICAL WASTEWATER TREATMENT

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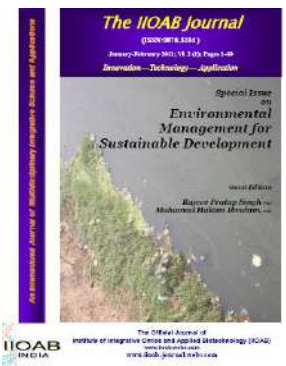
ABSTRACT



The wastewater generated from pharmaceutical industry generally contain high organic load and the treatment is primarily carried out using two major types of biological methods; aerobic and anaerobic. However, due to high strength, it is infeasible to treat some pharmaceutical wastewater using aerobic biological processes. As an alternative, an anaerobic process is preferred to remove high strength organic matter. Anaerobic wastewater treatment is considered as the most cost effective solution for organically polluted industrial waste streams. In particular the development of high rate systems, in which hydraulic retention times (HRT) are uncoupled from solids retention times (SRT), has led to a worldwide acceptance of anaerobic wastewater treatment. In this paper, literature on anaerobic digestion, anaerobic reactor technology and existing anaerobic treatment of pharmaceutical wastewater are presented. In addition, fate of pharmaceuticals in the environment was also discussed in brief. A case study of a laboratory investigation into the treatment of pharmaceutical wastewater containing the antibiotic Tylosin in an anaerobic reactor was also given. Specifically, it was determined whether the anaerobic reactor could be used as a pre-treatment system at an existing pharmaceutical production plant. The performance of the reactor treating real pharmaceutical wastewater at various organic loading rate (OLR) was investigated and showed efficient substrate removal at low OLRs (0.43 – 1.86 kg COD.m^{-3}.d^{-1}) by promoting efficient chemical oxygen demand (COD) reduction (70 – 75%). Under these conditions, an average of 95% Tylosin reduction was achieved in the UASR. However, increasing the OLRs to 3.73 kg COD.m^{-3}.d^{-1} by reducing the hydraulic retention time (HRT) (4 – 2 d) reduced the COD removal efficiency (45%). Changes in the organic loading affected the treatment performance of the anaerobic reactor, and at high OLRs, it was not able to withstand the short HRT, probably due to the complexity of pharmaceutical wastewater.

Keywords: *Anaerobic digestion; antibiotic; pharmaceutical wastewater; tylosin; UASR*

[I] INTRODUCTION

1.1. Anaerobic digestion

In the past, aerobic processes were very popular for biological treatment of wastewater in the 1960s. However, the energy predicament in the early 1970s brought about a significant change in the methodology of wastewater treatment. Energy preservation in industrial processes became a major concern and anaerobic processes rapidly emerged as an acceptable alternative. One of the important advantages of anaerobic digestion is the energy production during the process in the form of methane. Moreover, when high loading rates are accommodated, the area needed for the reactor is small. The sludge production is low, when compared to aerobic methods, due to the slow growth rates of anaerobic bacteria [1].

Figure–1 illustrates the advantage of anaerobic system in relation to aerobic treatment [2]. In aerobic process around 40 – 50% of biological stabilization take place, with its consequent

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conversion into CO_2. The sludge production and non degraded material in aerobic system is around 50 – 60% and 5 – 10%, respectively. However, in anaerobic system most of the biodegradable material is converted into biogas (around 70 – 90%), and only small portion of the organic material converted into sludge (about 5 – 15%). The material not converted into biogas leaves the reactor as non degraded material (around 10 – 30%). It is notable that the production of methane gas and the very low production of solids is the main advantage of anaerobic treatment.

Anaerobic wastewater treatment is considered as the most cost-effective solution for organically polluted industrial waste streams [3]. Toxic and recalcitrant wastewaters, that were previously believed not to be suitable for anaerobic processes, are now effectively treated. Accordingly, effluents from manufacturing operations in the pharmaceutical industry, such as antibiotic formulation, usually contain recalcitrant compounds. The following section discusses briefly the effluent from pharmaceutical industry.

1.2. Effluent from pharmaceutical industry

The pharmaceutical manufacturing industry produces a wide range of products to be used as human and animal medications. Manufacturing can be characterized by five main processes; fermentation, extraction, chemical synthesis, formulation and packaging [4]. Each of these steps may generate air emissions, liquid effluents and solid wastes**.** Liquid effluents resulting from equipment cleaning after batch operation contain toxic organic residues. Their composition varies, depending on the product manufactured, the materials used in the process, and other process details. Typically, pharmaceutical wastewater is characterized by high COD concentration, and some pharmaceutical wastewaters can have COD as high as 80,000 $mg.L^{-1}$[5]. Pharmaceuticals pose potential risks to the aquatic environment such as endocrine disrupting and side effects since they initially cause specific biological effects [6, 7]. Furthermore, wastewaters produced from antibiotic manufacture and formulation, generally contain high levels of soluble organics, many of which are recalcitrant [8]. If these compounds are not removed by one-site treatment they will be discharged to sewage treatment plants (STPs). This then eventually could disturb the biological process and the microbial ecology in the STP and the receiving surface waters [6, 7, 9–13].

Widespread work into the occurrence and fate of pharmaceuticals in the environment has been carried out in recent years [14–20]. The aim of the majority of this work has been to identify particularly persistent substances. In addition, the quantities in which they occur in surface waters and wastewater effluents and the eventual long-term effects they may have in the aquatic environment. Essentially, the detection of pharmaceuticals such as antibiotics in the environment has raised concern about potential human health effects. Pharmaceuticals can enter the aquatic environment through the sewage treatment systems when they are excreted by people, or if they are disposed in the home [21]. They can also enter sewage treatment works or watercourses as a result of discharges from pharmaceutical manufacturing plants or medical establishments. The degree of discharge from sewage treatment works depends on how they are affected by the treatment process.

1.3. Anaerobic treatment of pharmaceutical wastewater

Effluent from pharmaceutical wastewater normally treated using flocculation, flotation, coagulation, filtration, settling, ion exchange, carbon adsorption, detoxification of active ingredients by oxidation (using ozone wet air oxidation ultraviolet systems or peroxide solutions), and biological treatment (using trickling filters, anaerobic, activated sludge, and rotating biological contactors). Although pharmaceutical wastewater may contain refractory organic materials that cannot be readily degraded, biological treatment is still a viable choice for treatment [22, 23]. However, due to high strength, it is infeasible to treat some pharmaceutical wastewater using aerobic biological processes. Instead an anaerobic process is preferred to remove high-strength organic matter. Recently, the anaerobic treatment of pharmaceutical wastewater containing antibiotics and synthetic drug based effluents has been reported. The detail discussion on this can be found below.

Table–1 shows treatment of various pharmaceutical wastewater using anaerobic processes. Fox and Venkatasubbiah [24] have demonstrated the use of anaerobic baffled reactor (ABR) in the treatment of high sulphate containing pharmaceutical wastewater (Isopropyl Acetate fermentation). These workers found that by inserting a sulphide oxidation unit, the COD removal efficiency could be increased up to 50% at HRT 1 d. Massé *et al.* [25] have explored the effect of antibiotics on psychrophilic anaerobic digestion of swine manure slurry in sequencing batch reactors (SBRs). In their work, six antibiotics, Tylosin, Lyncomycin, Tetracycline, Sulphamethazine, Penicillin and Carbadox, were individually added to a pig diet. It is concluded that only Penicillin and Tetracycline had an inhibitory effect on methane production. Venkata Mohan *et al.* [26] have demonstrated the use of anaerobic suspended film contact reactor (ASFCR) in the treatment of pharmaceutical wastewater from large bulk drug manufacturing unit (aromatic and aliphatic organic chemicals). The organic loading rates were varied from 0.25 to 2.5 kg $COD.m^{-3}.d^{-1}$ and the COD reduction is in the range of 60 to 80% with methane content of around 60 - 70%. Nandy and Kaul [5] demonstrated anaerobic pre-treatment of herbal based pharmaceutical wastewater (e.g. herbs, fruits, flowers, roots, seeds, etc) using fixed-film reactor (FFR) and showed 76 – 98% COD removal at OLR of 10 kg $COD.m^{-3}.d^{-1}$. However, when the OLR increased to 48 kg $COD.m^{-3}.d^{-1}$, the COD removal efficiency dropped to 46 – 50%. They also found that the reactor did not show destabilization under hydraulic and organic shock loadings.

Saravanane *et al.* [27] has demonstrated that a fluidized bed reactor (FBR) under anaerobic conditions could be used to treat anti-osmotic drug based pharmaceutical effluent (Acetic acid and Ammonia). It is reported that COD reduction attained a maximum value of 88.5% using bioaugmentation through periodic addition of acclimated cells every 2 days with 30 - 73.2 g of cells (1 to 2.5 $g.L^{-1}$ of reactor volume) from an off-line enricher reactor. Furthermore, they also adventured into studying on bioaugmentation and treatment of Cephalexin drug based pharmaceutical effluent in an up-flow anaerobic fluidized bed (UAFB) system [28]. The results showed that bioaugmentation improved removal efficiency and reactor stability. Ince *et al.* [29] carried out a study on the performance of an up-flow anaerobic filter (UAF) treating a chemical synthesis-based pharmaceutical wastewater (Bacampicilline and Sultamicilline Tosylate) and showed 65% COD removal with methane yield being low at 0.20 m^3 $CH_4.kg$ COD_r^{-1}. The performance of a sequencing batch biofilter (SBB) integrating anaerobic-aerobic conditions in one tank to treat a pharmaceutical wastewater (Phenols and O-Nitroaniline) was studied by Buitron *et al.* [30]. The results showed that at HRT 8 – 24 h and OLR of 4.6 – 5.7 kg $COD.m^{-3}.d^{-1}$, a COD removal of 95 – 97% was achieved in the combined system. Anaerobic treatment of pharmaceutical wastewater (Penicillin) containing sulphate (3200 $mg.L^{-1}$) was carried out by Rodríguez-Martinez *et al.* [31] in an UASB and showed 85 - 90% COD and a sulphate removal of more than 90% were achieved at an OLR of 1.5 kg $COD.m^{-3}.d^{-1}$ and HRT of 8.3 d. However, the performance of the reactor was affected (COD removal dropped to 70%) when the loading rate was increased to 2.09 kg $COD.m^{-3}.d^{-1}$ by reducing the HRT to 7 d. The authors suggested that the accumulation of sulphides could be responsible for the reduced performance. Anaerobic-aerobic treatment of pharmaceutical containing antibiotics (Ampicillin and Aureomycin) was investigated by Zhou *et al.* [32] in an anaerobic baffled reactor (ABR) followed by a biofilm airlift suspension reactor (BASR). The combined system resulted in total COD removal of 97.8% when ABR and BASR were operated at HRT 2.5 d and 12.5 h, respectively. The Ampicillin and Aureomycin removal effiencies were 42.1% and 31.3% in the ABR, respectively, but did not show substantial removal (less than 10%) in BASR for both antibiotics. More recently, Oktem *et al.* [35] have conducted a study on the performance of a lab-scale hybrid up-flow anaerobic sludge blanket (UASB) reactor, treating a chemical synthesis-based pharmaceutical wastewater. At an OLR of 8 kg $COD.m^{-3}.d^{-1}$, COD reduction of 72% was achieved in the reactor system.

1.3. Treatment of pharmaceutical wastewater-a case study

In this section, a case study of the treatment of pharmaceutical wastewater containing the antibiotic Tylosin in an up-flow anaerobic stage reactor (UASR) is presented. Stage reactors can provide high treatment efficiency for recalcitrant substrates because phase separation, which generates separate environments for acidogenesis and methanogenesis, also promotes favourable conditions for microbial populations involved in the degradation of recalcitrant compounds.

Tylosin is a macrolide antibiotic produced by a strain of *Streptomyces fradiae*. It has good anti-bacterial activity against most pathogenic gram-positive bacteria, and some gram-negative bacteria, vibrio, spirochete, coccidian, etc. It is one of the first-choice drugs against infections caused by mycoplasma.

[II] MATERIALS AND METHODS

The UASR system [Figure–2] comprise four identical cylindrical Plexiglas compartments (stages), 80 mm internal diameter by 640 mm height, linked in series, was constructed for the present study. The active volume of the UASR system was 11 L (4 stages of 2.75 L). The operational set-up, flow diagram and the reactor design are presented in Figure–2a. Each stage of the reactor had a 3-phase separator baffle, angled at 45° and placed 50 mm below the effluent ports, to prevent floating granules from washing out with the effluent [Figure–2b]. The walls of the reactors were wrapped with a tubular PVC water-jacket, 15mm internal diameter, to maintain the reactor temperature at 37° C. Peristaltic pumps (Watson Marlow 100 series) were used to control the influent feed rate to the first stage of the UASR.

The pharmaceutical wastewater had the following characteristics; soluble COD, 7000 ± 800 $mg.L^{-1}$; soluble BOD_5, 3500 ± 500 $mg.L^{-1}$; sulphate, 2500 ± 500 $mg.L^{-1}$; Total Kjeldahl Nitrogen (TKN), 364 ± 50 $mg.L^{-1}$; pH, 5.2 – 6.8 and Tylosin concentration, 10 to 220 $mg.L^{-1}$. In general, this study was carried out in four major steps: 1) start-up of UASR, 2) acclimatisation to pharmaceutical wastewater, 3) increase in OLR (0.43 – 1.86 kg $COD.m^{-3}.d^{-1}$) by altering feed COD (1700 – 7450 $mg.L^{-1}$) at constant HRT (4 d), and 4) increase in OLR (2.48 – 3.73 kg $COD.m^{-3}.d^{-1}$) by reducing HRT (4 – 2 d) at constant feed COD (7450 $mg.L^{-1}$). Table–2 shows the reactor operating conditions during investigation of OLR on treatment process. Supernatant liquor, gas and sludge samples were taken separately from each stage for analysis. In addition, gas production rate was determined separately for each stage. Sample analysis included chemical oxygen demand (COD), pH, alkalinity, total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_3-N), suspended solids (SS), volatile suspended solids (VSS), all according to Standard Methods [39].

Tylosin assay was performed by HPLC on a 20cm Nucleosil C18 analytical column eluted with 60 vols 2 $mol.dm^{-3}$ sodium perchlorate ($NaClO_4$) and 40 vols of acetronitrile (CH_3CN). Tylosin factors were separated and detected at 280nm. The integrated chromatogram was normalised and the relative percentage of each Tylosin factor reported. Comparison of each Tylosin sample chromatogram with that of a Tylosin base reference standard chromatogram confirmed peak identity for quantification against a 3-point standard curve.

[III] RESULTS AND DISCUSSION

Figure–3 shows temporal changes in the total COD removal and fractional contribution by each stage of the UASR treating pharmaceutical wastewater. Initial fluctuations were attributed to technical problems with the peristaltic feed pump. At a reactor OLR of 1.86 kg $COD.m^{-3}.d^{-1}$ (HRT 4 d), the soluble COD reduction was around 70 - 75%. However, when the OLR was increased to 2.48 kg $COD.m^{-3}.d^{-1}$ (by lowering the HRT, since the strength of the wastewater was limited) the COD removal efficiency decreased gradually until only around 45% soluble COD removal (average removal when reactor approached steady-state) was observed at an OLR of 3.73 kg $COD.m^{-3}.d^{-1}$. It is unlikely that this was caused by limitations in the reactor design as similar ABR have been shown to be capable of over 90% COD removal at OLR of more than 10 kg $COD.m^{-3}.d^{-1}$ [40].

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However, pharmaceutical wastewaters containing a high proportion of spent fermentation broths have been shown to require long HRT for efficient treatment [41], presumably on account of their complex organic carbon content, and this is probably limits the UASR performance at HRT below 4 d.

Table: 1. Anaerobic treatment of pharmaceutical wastewater

Anaerobic Reactor	Type of Pharmaceutical Wastewater	COD Removal (%)	References
Fixed bed	Phenol	93	Bajaj et al [38]
Periodic baffled system	Chinese traditional medicine	34 - 84	Liu et al [37]
Hybrid up-flow sludge bed	Phenol, Dibutyl Phthalate, Bromo Naphthalene, Carbamazepine, Antipyrine	65 - 75	Sreekanth et al [36]
Hybrid up-flow sludge bed	Chemical synthesis	72 – 85	Oktem et al [35]
Up-flow sludge bed	Antibiotic formulation (sulfamerazine)	68 - 89	Sponza and Demirden [34]
Sequencing batch bio-film	Chemical / bulk drugs	51	Venkata Mohan et al [33]
Baffled system	Antibiotic formulation (Ampicillin, Aureomycin)	77 - 90	Zhou et al [32]
Up-flow sludge bed	Antibiotic formulation (Penicillin)	90	Rodriguez-Martinez et al [31]
Sequencing batch	Phenols and O-Nitroaniline	95 - 97	Buitrón et al [30]
Up-flow filter	Chemical synthesis	65	Ince et al [29]
Fluidized bed	Cephalexin drug, anti-osmotic drug	88.5	Saravanane et al [27, 28]
Fixed-film fixed-bed	Herbal-based	76 – 98	Nandy and Kaul [5]
Suspended film contact	Bulk drug (aromatic, aliphatic)	60 – 80	Venkata Mohan et al [26]
Sequencing batch	Swine manure slurry containing antibiotics	80	Massé et al [25]
Baffled system	Isopropyl Acetate	50	Fox and Venkatasubbiah [24]

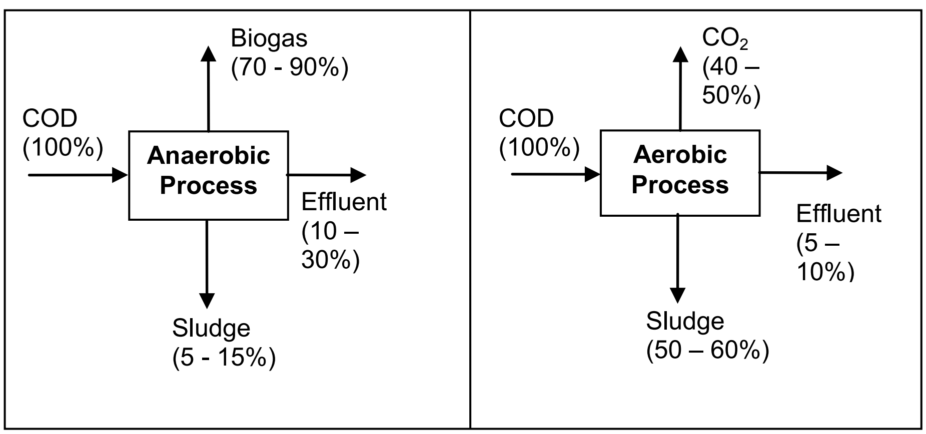

Fig: 1. Advantage of anaerobic system in relation to aerobic treatment [2].

The above results are consistent with observations made by Rodriguez-Martinez *et al.* [31] in an UASB treating pharmaceutical wastewater containing Penicillin G macrolide antibiotics, who found that the COD removal efficiency was 90% at an OLR of 1.5 kg $COD.m^{-3}.d^{-1}$ and HRT 11 d. However, when the OLR was increased to 2.09 kg $COD.m^{-3}.d^{-1}$ by reducing the HRT to 7 d, the COD removal efficiency dropped dramatically to 70%. They also found that an increase in the OLR resulted in the accumulation of hydrogen sulphide (sulphate in the feed was 3200 $mg.L^{-1}$) which affected the efficiency of the reactor; the presence of sulphide is known to inhibit the activity of methanogens [42].

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Table: 2. Summary of reactor operating conditions during investigation of OLR on treatment process

Brewery (%)* wastewater	Pharmaceutical (%)* wastewater	Mean OLR (kg COD.m^{-3}.d^{-1})	HRT (d)	Mean Feed COD (mg.L^{-1})	Day
50	50	0.43	4.0	1700	1
40–10	60–90	0.86	4.0	3450	41
0	100	1.23	4.0	4900	82
0	100	1.53	4.0	6100	109
0	100	1.86	4.0	7450	166
0	100	2.48	3.0	7450	188
0	100	2.98	2.5	7450	212
0	100	3.73	2.0	7450	231
0	100	1.86	4.0	7450	250

*proportion based on COD

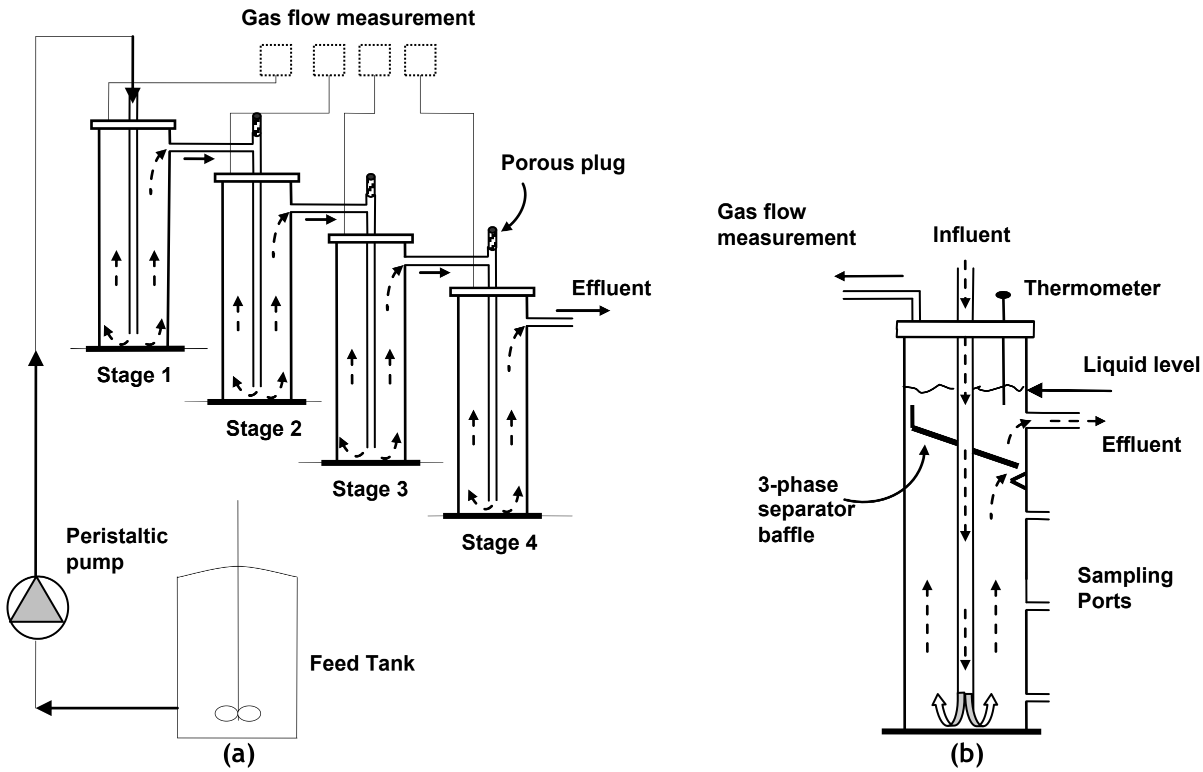

Fig: 2. (a) UASR system and flow regime; (b) details of an individual UASR stage

It is generally known the application of anaerobic treatment process for industrial wastewaters containing high amounts of sulphate has been problematic due to the production of hydrogen sulphide. The presence of H_2S in anaerobic digesters results from the action of sulphate-reducing bacteria (SRB) which utilise sulphate as terminal electron acceptor and compete with acetogens and methanogens for several key substrates in anaerobic digestion such as propionate, butyrate, ethanol and acetate [43]. Moreover, SRB are generally expected to out-compete other anaerobes in the presence of excess sulphate [44]. The pharmaceutical wastewater used in this study contained high amount of sulphate and sulphide production from this sulphate was thought to be one of the reasons for the poor performance of UASR during the period of high OLR (2.48 – 3.73 kg COD.m^{-}

^{3}.d^{-1}). Speece, [45] has stated that at higher OLR, SRB can out-compete with methanogens for substrate since hydrogen sulphide production can be predominant over methane gas production. Kuscu and Sponza, [46] have demonstrated that hydrogen sulphide concentrations in the gas were increased from 160 mg.L^{-1} to 195 mg.L^{-1} when OLR was increased from 2.1 to 3.16 kg COD.m^{-3}.d^{-1} in an ABR treating sulphate containing wastewater (*p*-Nitrophenol). Consequently, the decrease in treatment efficiency in the UASR was probably due to sulphide inhibition at higher OLRs (2.48 – 3.73 kg COD.m^{-3}.d^{-1}).

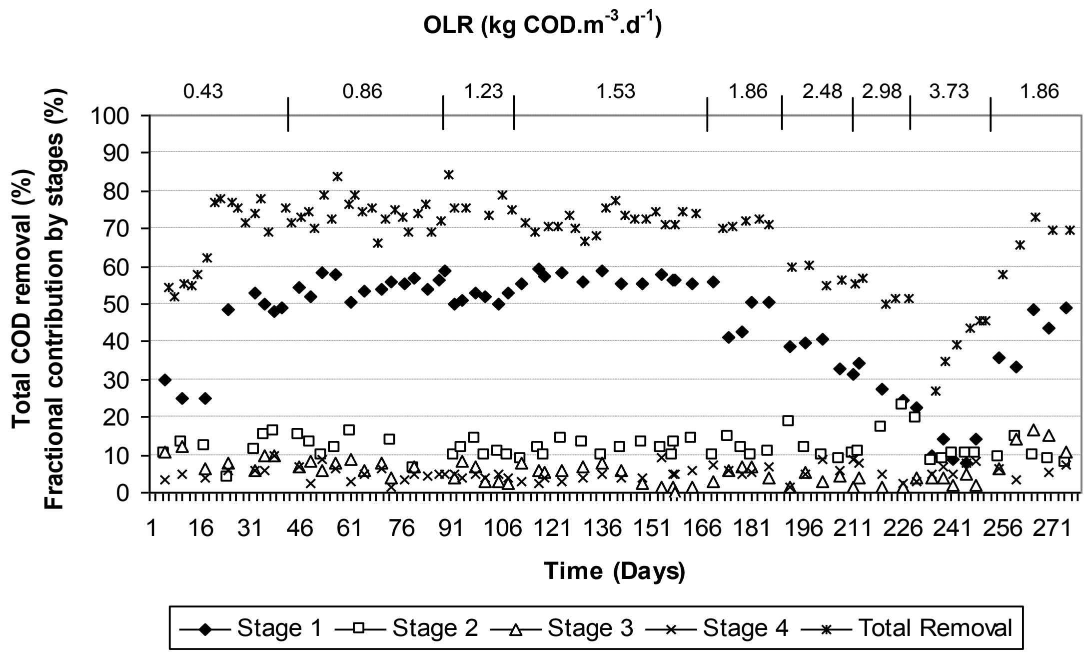

Fig: 3. Total COD reduction (%) of UASR treating pharmaceutical wastewater and fractional contribution (%) to the total COD reduction by each stage at different OLR.

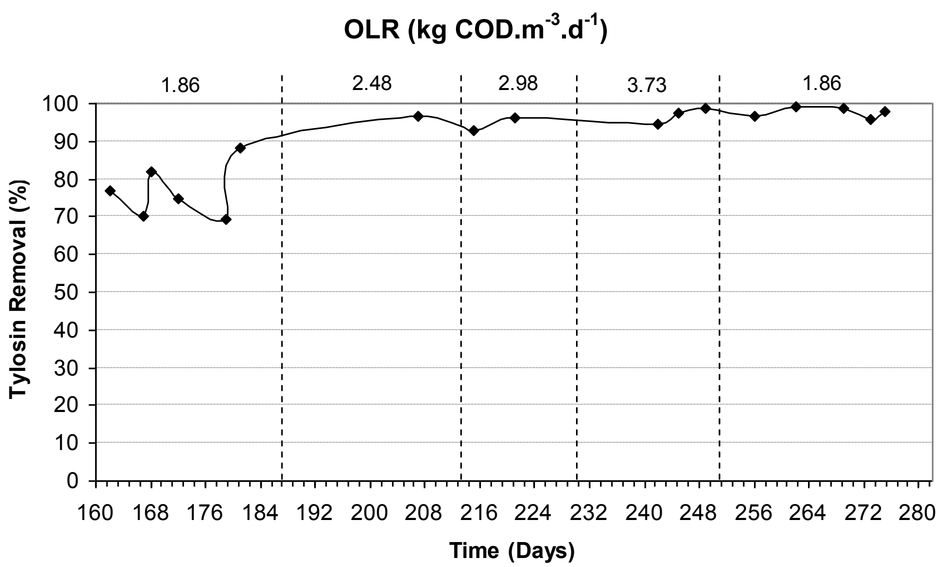

Fig: 4. Antibiotic (Tylosin) reduction profile of UASR at different selected OLR

Fox and Venkatasubbiah [24] reported that as influent pharmaceutical wastewater containing high sulphate was increased to 20% in an ABR, the reactor performance deteriorated (COD removal efficiency reduced from 50 to 20%) as the effluent sulphide concentration increased to inhibitory levels (more than 200 $mg.L^{-1}$). In addition, Nandy and Kaul [5] have demonstrated that substrate removal efficiency increases with increase in HRT in anaerobic treatment of herbal-based pharmaceutical wastewater using fixed-bed reactor. More recently, Zhou *et al*. [32] reported that when HRT of an ABR treating pharmaceutical wastewater containing antibiotics (Ampicillin and Aureomycin) was extended from 1.25 to 2.5 d, the COD removal efficiency increased from 77 to 85%. They also observed that the antibiotic removal efficiencies increased from 16 to 42% for Ampicillin and 26 to 31% for Aureomycin.

It is evident that stages 2, 3 and 4 showed a relatively minor contribution to total COD removal, around 50 to 60% COD reduction took place in Stage 1 of the UASR when reactor HRT was set to 4 d (i.e. for all reactor OLR at or below 1.86 kg $COD.m^{-3}.d^{-1}$), with less contribution from Stage 2 (around 10 - 15%), and Stage 3 and 4 accounting for around 5%. This also suggests that it was the physiological characteristics of the Stage 1 effluent that limited further COD degradation in subsequent stages of the reactor, rather than excessive OLR, although as the pH was reduced in all stages at the highest OLR (data not presented), there is a possibility that the methanogenic biomass in Stages 2, 3 and 4 could also have been affected adversely by the acidic conditions generated in Stage 1. Another possible reason could be the sulphide toxicity at higher OLRs in Stage 1 which inhibited the methanogens in Stage 2, 3 and 4. Moreover, the increase in OLR (by decreasing in HRT) had a greater adverse effect on COD degradation efficiency than increases in substrate concentration at a fixed HRT. In UASR, the decrease in HRT decreased treatment efficiency, especially in Stage 1, and since other stages were not working effectively, the overall treatment efficiency is low.

In this study, Tylosin concentration in the pharmaceutical wastewater feed varied from 10 to 220 $mg.L^{-1}$ and **Figure–4** shows the Tylosin degradation profile throughout the experimental study in the UASR. Tylosin removal efficiency fluctuated from 70 – 88% at OLR 1.86 kg $COD.m^{-3}.d^{-1}$, however, the removal efficiency remained relatively constant (93 – 99%) at OLR 2.48 - 3.73 kg $COD.m^{-3}.d^{-1}$. Similar removal trend was also observed when the reactor OLR was reduced to 1.86 kg COD m^{-1} d^{-1} **[Figure–4]**, with an average Tylosin concentration in the treated wastewater of 3 $mg.L^{-1}$ for the all OLR investigated. This confirms that Tylosin was readily degraded in the reactor under anaerobic conditions. In contrast to the COD removal profile, which showed reducing COD removal efficiency with increasing OLR, Tylosin concentration remained relatively constant in the reactor effluent throughout the experiment. These results are consistent with the view that typical wastewater concentrations of Tylosin have a relatively minor influence on the overall COD removal efficiency of UASR and do not inhibit substantially the activity of methanogenic populations. Some may argue Tylosin is hardly biodegradable and could contribute to high COD in the effluent; however, we believe, the anaerobic treatment system (UASR) operated to efficiently remove most of the general COD associated with fermentation waste residues in the real pharmaceutical wastewater containing Tylosin. Further polishing by aerobic degradation would be viable if tight discharge consent applied (i.e. aerobic polishing after anaerobic digestion process is better than using aerobic to degrade all COD).

[IV] CONCLUSIONS

Anaerobic treatment system is a promising alternative for pharmaceutical wastewater treatment. Results from the existing treatment of pharmaceutical wastewater using anaerobic system demonstrates that anaerobic treatment is suitable for treating various type of pharmaceutical wastewater. The application of anaerobic digestion to recalcitrant streams such as those from pharmaceutical production would provide significant environmental and economic benefits to pharmaceutical industry. The UASR system is an appropriate option for pre-treatment of wastewaters with a highly complex organic composition, such as pharmaceutical wastewater. Results of this study suggest that at a reactor OLR of 1.86 kg $COD.m^{-3}.d^{-1}$ (HRT 4 d); the soluble COD reduction was around 70 - 75%. Under these conditions, an average of 95% Tylosin reduction was achieved in the, indicated that this antibiotic could be degraded efficiently in the anaerobic reactor system. However, when the OLR was increased to 2.48 - 3.73 kg $COD.m^{-3}.d^{-1}$, by lowering the HRT, the COD removal efficiency decreased to 45%. Whilst COD degradation efficiency might be affected by the complexity and variability of the real pharmaceutical wastewater, long HRT in the UASR can lessen these effects.

FINANCIAL DISCLOSURE

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RESEARCH ARTICLE

MANAGEMENT OF HEAVY METAL CONTAMINATED SOIL BY USING ORGANIC AND INORGANIC FERTILIZERS: EFFECT ON PLANT PERFORMANCE

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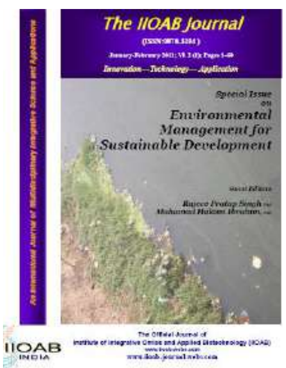
ABSTRACT



Heavy metal contamination leads to variety of harmful effects on soil and plant characteristics. In order to reduce the toxic effects of such substances, an experiment was conducted by FYM, N, NPK, FYM + NPK and FYM + N amendments in the soil from an area irrigated by waste water for more than 20 years. Soil and plant characteristics were compared between fertilizer (FYM, NPK, N and FYM + N, FYM + NPK) amended and non-amended control soil. As compared to the control, plants under FYM and FYM + NPK amendments showed lower accumulation of heavy metals and higher yield. Plants grown in NPK and N amended soil showed higher concentrations of heavy metals and lower yield compared to the control. Higher uptake of heavy metals in plants under NPK and N amendments, led to increase in the antioxidants enzymes, but reductions in photosynthesis rate, growth and yield. The results suggest that the application of FYM alone and in combination with inorganic fertilizers may be recommended as cost effective technique for reducing the availability of heavy metals in waste water irrigated soil.

Keywords: *heavy metals; contamination; fertilizer; remediation; soil; plant*

[I] INTRODUCTION

Pollution of the biosphere by toxic metals has accelerated severely since the beginning of the industrial revolution. The primary sources of metal pollution include the burning of fossils fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and waste water irrigation. Contamination of groundwater and soil by heavy metals leads to major environmental and human health problems. Plant metabolism is also affected negatively by the heavy metals [1]. Although some of the heavy metals act as micronutrient at lower concentration, but at higher concentration these are harmful for the normal functioning of plants [2]. Metals cannot be degraded to harmless products, such as carbon dioxide, but instead persist indefinitely in the environment, complicating their remediation.

Occurrence of oxidative stress in plants could be the indirect consequence of heavy metal toxicity [3, 4]. Molecular oxygen can accumulate in the leaves of plants under heavy metal stress, which can result into oxidation of cellular components. One of the most deleterious effects induced by heavy metals exposure in plant is lipid peroxidation, which can directly cause biomembrane deterioration [5]. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membrane is regarded as a reliable indicator of oxidative stress [6]. In response to generation of reactive oxygen species, plants induce the enzymatic and non enzymatic antioxidants that have ability to detoxify these species [7].

Exposure of plants to heavy metals causes reduction in photosynthesis, water uptake, and nutrient uptake [8]. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips, and finally death [9, 10].

An effective and affordable technological solution is needed for marginal farmers to reduce the harmful effects of heavy metals in the area using waste water for irrigation. The practices that improve soil quality and sustainability of agricultural system may be the most important acceptable technique. In the present study, the amendments of organic and inorganic fertilizers alone and in combination in the soil was done to reduce the availability of heavy metals in the waste water irrigated soil and the consequent effects on heavy metal availability, biochemical, physiological, growth and yield responses of palak (*Beta Vulgaris L.*) were assessed.

[II] MATERIALS AND METHODS

2.1. Experimental site

The experiment was conducted in Dinapur situated at a suburban area in the north east of Varanasi (25º18' N latitude 83º01' E longitude and 76.19 m above the mean sea level) city in the eastern Gangetic plains of India, having long term uses of treated and untreated waste water for irrigation from Dinapur sewage treatment plant (DSTP) of 80 million liters per day capacity.

2.2. Field preparation and raising of plants

Experimental pots (radius = 12.5 cm) were prepared according to common agronomic practices. Soil was amended with farmyard manure (FYM) at 80 t ha^{-1}, urea (N) + superphosphate (P) + potash (K) at 80:40:40 kg ha^{-1}, respectively only urea (N) at 80 kg $ha^{-1}$and a combination of FYM (80 t ha^{-1}) + urea (80 kg ha^{-1}), and FYM at 80 t ha^{-1}+ NPK at 80:40:40 kg ha^{-1}, respectively. Control pots (C) were kept without any amendment. There were 10 replicate pots for each amendment. Genetically uniform seeds of palak (*Beta vulgaris* L. All green) obtained from Institute of Vegetable Research, Varanasi. Three seed were sown in the pot at 2 cm depth in triangle at equal distance. Waste water from DSTP was used for irrigating all the pots including control. After germination, only one plant was left in each pot.

2.3. Soil Sampling and preparation

Soil samples were collected in triplicate from pots of each amendment, dried at room temperature, crushed, sieved with 2 mm mesh size sieve and kept at room temperature for further analysis.

2.4. Sampling of plants

Plants were dug out along with intact root at random from each pot for various growth and yield analysis at the time of harvest i.e. 40 days after germination (DAG). These were thoroughly washed by placing them on a sieve of 2 mm mesh size under running tap water to remove soil particles adhering to the roots.

2.5. Soil analyses

Soil pH was measured in suspension of 1:5 (soil: water w/v) using a glass electrode standardized with pH 4, 7 and 9.2 buffer tablets attached to an Ion analyzer (Model E.A 940, Orion USA). The organic carbon content was determined by using modified Walkley and Black's rapid titration method [11].

The total nitrogen (TN) content was determined by following the micro-Kjeldahl technique through the Gerhardt automatic analyzer (Model KB8S, Kjeldatherm, Germany). Available phosphorus ($NaHCO_3$ extractable) was determined by the method given by Allen et al. [12]. Exchangeable cations such as Na, K, Mg, Fe and Ca were extracted using ammonium acetate solution through repeated leaching technique (Jackson 1958), and contents were determined by atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

2.6. Digestion and analysis for heavy metals

Soil and plant samples (1g) were digested by adding tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio) at 80 °C until a transparent solution was obtained [12]. After cooling, the digested sample was filtered using Whatman no. 42 filter paper and the filtrate was finally maintained to 50 ml with distilled water. Concentrations of heavy metals in the filtrate of digested soil and plant samples were estimated by using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA) fitted with specific lamp of particular metal using appropriate drift blank.

Phytoavailable heavy metals in the soil samples were extracted by the method given by Quevauviller et al. [13]. Sieved soil sample of 10 g was shaked with 20 ml of 0.05 N EDTA solution (pH = 7) for 1 hour and then kept for 24 hours before filtering. The concentrations of phytoavailable heavy metals in the filtrate were determined by using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

2.7. Physiological characteristics

Portable Photosynthetic System (Model 6200, LICOR, Lincolin, NE, USA) was used for measuring photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency at ambient climatic conditions on intact plants in the pot. The system was calibrated using a known CO_2 source of 509 ppm concentration. These parameters were measured between 9.00 and 10.00 hours on cloud free days. During the measurements, photosynthetically active radiation ranged between 1100 to 1200 µmol m^{-2} s^{-1}.

Measurement of chlorophyll fluorescence was done with the help of Plant Efficiency Analyzer (Hansatech Instruments Ltd., England, PEA MK2, 9414) between 10.00 to 11.00 hours on the same foliage where photosynthetic rate was measured. Before taking measurements, leaves were dark adapted for 30 minutes. The adaxial surface of leaf was irradiated with red light and fluorescence signal was collected from the same surface. Chlorophyll fluorescence characteristics such as initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v = F_m - F_o) and F_v/F_m ratio were measured for different treatments.

2.8. Biochemical characteristics

Fresh plant leaves from three different pots were sampled at 40 Days after germination (DAG) for estimation of photosynthetic pigments, lipid peroxidation and different metabolites and enzymes. Ascorbic acid and proline contents were determined by the methods described by Keller and Schwager [14] and Bates et al. [15], respectively. Total phenol content was measured using the method of Bray and Thorpe [16]. The methods of Britton and Mehley [17] and Fahey et al. [18] were used for analyzing peroxidase activity and thiol content, respectively. The lipid peroxidation was measured as Malondialdehyde (MDA) concentration by following the protocol given by Heath and Packer [19].
Chlorophyll (Chl) and carotenoids were extracted with 80 % acetone and the amounts were estimated spectrophotometrically by the method of Machlachlan and Zalick [20] and Duxbury and Yentsch [21], respectively.

2.9. Growth parameters and yield of plant

Growth parameters were analysed with respect to root and shoot lengths, number of leaves per plant and leaf area. The leaf area was measured with the help of LI-COR leaf area meter (Model LI-COR 3000, LI-COR Inc., NE, USA). For biomass estimation, different plant parts were separated and oven-dried at 80°C till a constant weight was obtained. Oven-dried plant parts were weighed separately for biomass estimation.

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At 40 DAG, 10 plants of each treatment were harvested and yield was calculated as fresh weight of the edible part of the plant.

2.10. Statistical analysis

The data under different amendments were subjected to ANOVA test for assessing the significance of differences in heavy metal concentrations, biochemical, physiological and growth parameters and yield of plants. All the statistical tests were performed using SPSS software (SPSS Ins., version 12).

2.11. Quality control analysis

Precision and accuracy of analysis was assured through repeated analysis of samples against National Institute of Standard and Technology, Standard Reference Material (SRM 1570) for all the heavy metals. The results were found within ± 2 % of the certified value. Quality control measures were taken to assess contamination and reliability of data. Blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run after five determinations to calibrate the instrument. The coefficients of variation of replicate analysis were determined for different determinations for precision of analysis and variations were found to be less than 10 %.

[III] RESULTS AND DISCUSSION

3.1. Physico-chemical properties of soil

Among all the amendments, pH was maximum for FYM and minimum for NPK amendments in the soil at both the samplings time **[Table–1, –2]**. Soil pH is one of most important characteristic for determining the availability of heavy metals. Application of FYM increases the organic matter of soil that consequently increases the pH of soil due to the release of fumic and humic acid through the process of decomposition. Mgbeze and Abu [22] have also found higher pH in river sand amended with FYM compared to the control at Ugbowo campus of the University of Benin, Nigeria. Application of NPK showed lowest pH because phosphate increases the formation of slowly soluble Dicalcium phosphate (DCP) with a release of phosphoric acid which is responsible for decreasing the soil pH through its dissociation in to phosphorus and acidic hydrogen ion. Zheng Miao et al. [23] have also found lower soil pH under the application of super phosphate fertilizers.

Higher concentration of NO_3^-- N and NH_4^+- N in N amended soil was found at both the samplings **[Table–1, –2]**. Soil amended with N alone showed 36, 33, 55, 28, 29 % increments in NO_3^-- N concentration as compared to the control, NPK, FYM, FYM + NPK and FYM + N amended soil, respectively at the final sampling **[Table–2]**. Higher concentration of available forms of N (NO_3^-- N and NH_4^+- N) in N amended soil is due to the application of urea alone. Highest concentration of available P was found in NPK amended soil compared to other amendments at both initial and final sampling due to addition of phosphate fertilizer **[Table–2]**.

At both the samplings, FYM amended soil showed highest value of organic C and NPK amended soil showed lowest. At initial and final samplings, respectively, organic C increased maximally by 21 and 16 % in FYM amended soil and decreased by 8 and 9 % in NPK amended soil compared to the control soil **[Table–2]**. Application of organic matter in the form of FYM enhanced the organic C level of the soil and has direct and indirect effects on soil properties and processes [24]. Ruhlmann and Ruppel [25] have also found FYM to be a good source of organic matter.

Among different amendments, concentrations of exchangeable cations were highest in soil amended with FYM alone followed by the combination of FYM with inorganic fertilizer and least in those amended with inorganic fertilizer alone and control soil at the both the samplings **[Table–1, –2]**. Bhattacharyya et al. [26] have also found that FYM amended plots showed better supply of N, P, and K, and improved soil physical conditions compared to unamended ones. Soil amended with FYM + NPK showed higher concentrations of cations because the decomposition products of FYM in soil have arrested the fixation/adsorption of the applied fertilizer and resulted in to higher availability of these nutrients [27].

Table: 1. Physico–chemical properties of soil after various amendments at initial sampling (Mean ± 1SE)

Properties	Control	FYM	NPK	N	FYM + NPK	FYM + N
pH	$7.16^b \pm 0.01$	$7.60^a \pm 0.11$	$6.90^c \pm 0.01$	$7.17^b \pm 0.10$	$7.27^b \pm 0.28$	$7.33^b \pm 0.23$
Conductivity (ds cm^{-1})	$0.13^a \pm 0.01$	$0.14^a \pm 0.03$	$0.13^a \pm 0.005$	$0.12^a \pm 0.003$	$0.14^a \pm 0.005$	$0.13^a \pm 0.005$
Total N (%)	$0.23^a \pm 0.01$	$0.21^a \pm 0.01$	$0.25^a \pm 0.01$	$0.26^a \pm 0.01$	$0.24^a \pm 0.003$	$0.25^a \pm 0.003$
NO_3^- – N (µg g^{-1})	$13.0^b \pm 0.57$	$11.67^c \pm 0.88$	$13.33^b \pm 0.67$	$15.0^a \pm 0.57$	$14.0^{ab} \pm 1.15$	$13.67^b \pm 0.88$
NH_4^+– N (µg g^{-1})	$4.67^b \pm 0.17$	$4.33^c \pm 0.17$	$5.17^b \pm 0.16$	$5.50^a \pm 0.29$	$5.00^b \pm 0.29$	$5.10^b \pm 0.21$
Organic C (%)	$2.30^c \pm 0.12$	$3.17^a \pm 0.17$	$2.10^c \pm 0.06$	$2.17^c \pm 0.14$	$2.87^b \pm 0.08$	$2.83^b \pm 0.12$
Available P (µg g^{-1})	$87.53^b \pm 1.44$	$83.33^b \pm 1.67$	$90.10^a \pm 2.39$	$81.67^b \pm 1.67$	$89.33^b \pm 2.33$	$87.33^b \pm 1.45$
Na (µg g^{-1})	$158.67^b \pm 6.96$	$172.0^a \pm 3.05$	$168.0^b \pm 5.29$	$165.3^b \pm 2.96$	$171.0^{ab} \pm 3.78$	$170.67^{ab} \pm 2.33$
(µg g^{-1})	$175.0^a \pm 4.04$	$181.0^a \pm 3.21$	$178.60^a \pm 0.88$	$177.67^a \pm 4.33$	$184.0^a \pm 3.51$	$182.30^a \pm 3.38$
Ca (µg g^{-1})	$528.34^b \pm 15.89$	$582.60^a \pm 16.34$	$512.0^b \pm 14.04$	$539.56^b \pm 9.84$	$576.0^a \pm 14.0$	$576.30^a \pm 18.44$
Mg (µg g^{-1})	$64.30^b \pm 4.26$	$74.67^a \pm 4.37$	$65.67^b \pm 2.96$	$62.20^b \pm 3.92$	$75.67^a \pm 3.38$	$75.0^a \pm 5.04$
Fe (µg g^{-1})	$124.35^a \pm 8.68$	$148.0^a \pm 7.57$	$138.34^a \pm 4.41$	$139.40^a \pm 8.29$	$129.20^a \pm 4.67$	$130.0^a \pm 8.08$

Different letters in each row showed significant difference at $p \leq 0.05$

Table: 2. Physico–chemical properties of soil under various amendments at the time of harvest of palak (Mean ± 1SE)

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Properties	Control	FYM	NPK	N	FYM + NPK	FYM + N
pH	$6.93^{bc} \pm 0.06$	$7.83^{a} \pm 0.17$	$6.76^{c} \pm 0.14$	$6.99^{b} \pm 0.17$	$7.13^{b} \pm 0.12$	$7.27^{b} \pm 0.12$
Conductivity (ds cm^{-1})	$0.15^{a} \pm 0.01$	$0.16^{a} \pm 0.003$	$0.15^{a} \pm 0.01$	$0.15^{a} \pm 0.003$	$0.16^{a} \pm 0.005$	$0.15^{a} \pm 0.005$
Total N (%)	$0.25^{a} \pm 0.01$	$0.28^{a} \pm 0.01$	$0.27^{a} \pm 0.01$	$0.30^{a} \pm 0.03$	$0.29^{a} \pm 0.03$	$0.27^{a} \pm 0.02$
NO_3^- – N (μg g^{-1})	$11.0^{b} \pm 0.57$	$9.67^{b} \pm 0.88$	$11.30^{b} \pm 0.60$	$15.0^{a} \pm 0.58$	$11.67^{b} \pm 1.20$	$11.60^{b} \pm 0.80$
NH_4^+ – N (μg g^{-1})	$2.33^{b} \pm 0.16$	$2.16^{b} \pm 0.10$	$2.50^{b} \pm 0.28$	$2.83^{a} \pm 0.44$	$2.67^{b} \pm 0.17$	$2.66^{b} \pm 0.16$
Organic C (%)	$2.30^{b} \pm 0.11$	$2.67^{a} \pm 0.17$	$2.10^{c} \pm 0.05$	$2.27^{b} \pm 0.15$	$2.37^{b} \pm 0.08$	$2.33^{b} \pm 0.12$
Available P (μg g^{-1})	$81.67^{b} \pm 1.67$	$75.67^{c} \pm 1.76$	$83.0^{a} \pm 1.52$	$76.67^{c} \pm 1.76$	$77.0^{c} \pm 2.08$	$79.33^{b} \pm 3.17$
Na (μg g^{-1})	$138.60^{b} \pm 6.9$	$152.0^{a} \pm 3.05$	$148.0^{c} \pm 5.23$	$145.30^{c} \pm 2.90$	$154.0^{ab} \pm 3.08$	$150.57^{ab} \pm 2.00$
K (μg g^{-1})	$153.0^{a} \pm 4.00$	$159.00^{a} \pm 3.00$	$156.56^{a} \pm 0.80$	$155.64^{a} \pm 4.03$	$162.0^{a} \pm 3.05$	$160.30^{a} \pm 3.30$
Ca (μg g^{-1})	$505.3^{b} \pm 15.8$	$559.60^{a} \pm 16.00$	$489.0^{c} \pm 14.40$	$516.60^{b} \pm 9.0$	$553.0^{ab} \pm 14.10$	$553.30^{ab} \pm 18.0$
Mg (μg g^{-1})	$79.67^{b} \pm 5.48$	$86.0^{a} \pm 3.51$	$70.0^{c} \pm 1.15$	$66.33^{c} \pm 3.18$	$80.0^{ab} \pm 2.88$	$83.0^{ab} \pm 0.57$
Fe (μg g^{-1})	$139.6^{a} \pm 3.17$	$158.30^{a} \pm 7.76$	$146.60^{a} \pm 1.67$	$156.0^{a} \pm 11.0$	$138.30^{a} \pm 4.41$	$139.30^{a} \pm 5.81$

Different letters in each row showed significant difference at $p \leq 0.05$

3.2. Heavy metal concentrations in the soil

At initial sampling, total concentrations of heavy metal in the soil did not show significant difference among different amendments, whereas phytoavailable metals showed their minimum concentrations in FYM amended soil **[Figure–1a, –1b]**. There were 32, 47, 42, 21, 24, 47 and 38 % reductions in phytoavailable concentrations of Cd, Cu, Pb, Zn, Mn, Ni and Cr, respectively in FYM amended soil compared to the control soil **[Figure–1a, –1b]**. Phytoavialability of heavy metals depend upon various physico-chemical properties of the soil, such as pH and organic C [28]. Soil amended with the FYM showed more reduction in availability of heavy metals because of high organic C content of the soil. Organic C present in the FYM is responsible for the release of negatively charged ions that attract the positively charged heavy metals and consequently results into more retention of heavy metals in the soil with lower availability to the plant [29]. Singh et al. [30] have also found FYM application to the soil to be an effective measure for reducing Cr toxicity to spinach plants in Cr–contaminated soils at farm Punjab Agricultural University (PAU), Punjab, India. At final sampling the total concentrations of all heavy metals were higher in FYM amended soil **[Figure–2]**. This may be due to more retention of heavy metals in the soil under FYM amendment.

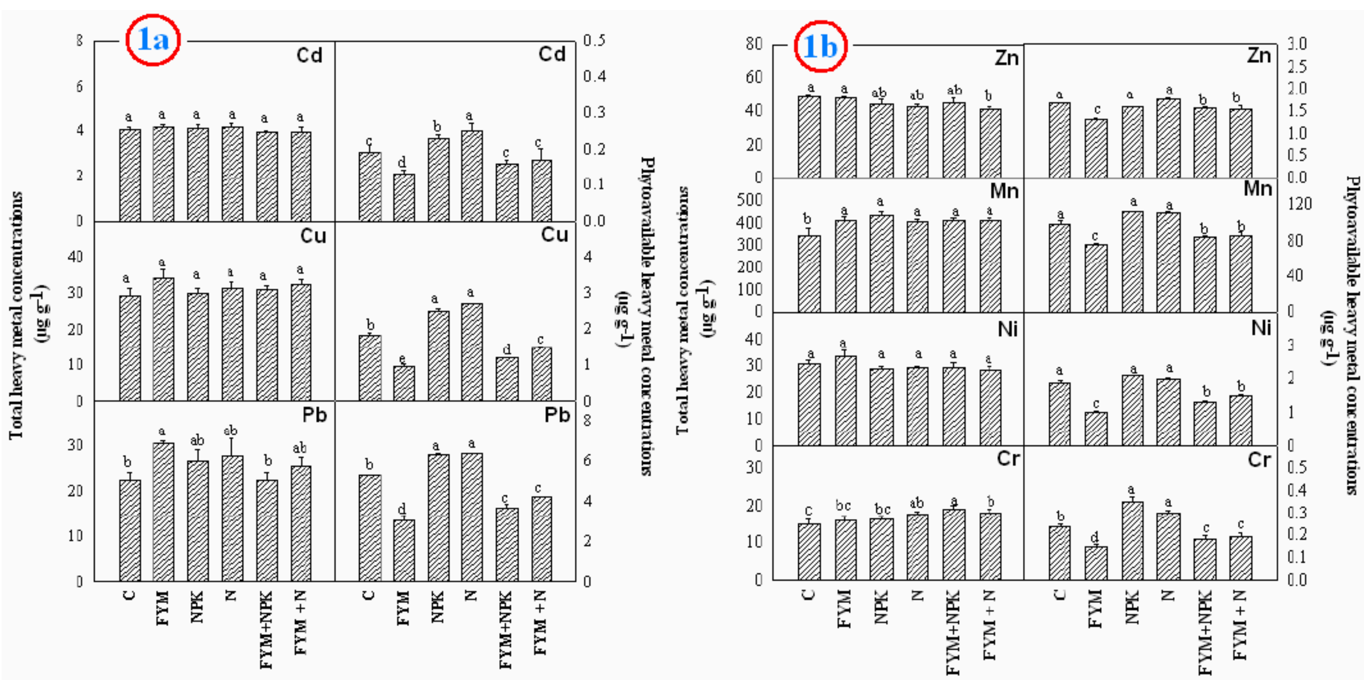

Fig: 1. (a) Total and phytoavailable concentrations of Cd, Cu and Pb in the soil at the time of initial sampling. (b). **Total and phytoavailable concentrations of Zn, Mn, Ni and Cr in the soil at the time of initial sampling**. Values are mean ± 1 SE. Bars with different letters in each group showed significant difference at p ≤ 0.05.

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Table: 3. Morphological characteristics and yield of palak at the time of harvest (Mean ± 1 SE)

Characteristics	Control	FYM	NPK	N	FYM + NPK	FYM + N
Root length (cm $plant^{-1}$)	12.98^{a} ± 0.88	11.07^{b} ± 0.71	13.55^{a} ± 0.86	13.55^{a} ± 0.72	11.60^{b} ± 0.79	11.05^{b} ± 0.68
Shoot length (cm $plant^{-1)}$	9.81^{c} ± 0.62	14.24^{a} ± 0.72	11.18^{c} ± 0.57	11.20^{c} ± 0.77	13.11^{b} ± 0.88	12.75^{b} ± 0.52
Number of leaves ($plant^{-1}$)	10.90^{b} ± 1.37	13.70^{a} ± 1.17	9.00^{c} ± 0.58	8.00^{c} ± 0.75	12.60^{b} ± 1.92	12.40^{b} ± 1.19
Leaf area ($cm^2 plant^{-1}$)	76.48^{c} ± 7.78	141.75^{a} ± 12.6	86.62^{c} ± 13.28	82.08^{c} ± 9.39	112.94^{b} ± 17.6	106.76^{b} ± 9.72
Shoot biomass (g $plant^{-1}$)	0.80^{c} ± 0.02	1.47^{a} ± 0.05	0.99^{c} ± 0.06	0.83^{c} ± 0.03	1.16^{b} ± 0.05	1.13^{b} ± 0.08
Root biomass (g $plant^{-1}$)	0.06^{a} ± 0.002	0.09^{a} ± 0.002	0.08^{a} ± 0.001	0.07^{a} ± 0.001	0.08^{a} ± 0.001	0.09^{a} ± 0.07
Yield (g $plant^{-1}$)	2.99^{b} ± 0.13	3.43^{a} ± 0.15	2.97^{b} ± 0.14	2.88^{b} ± 0.015	3.24^{ab} ± 0.20	3.13^{ab} ± 0.17

Different letters in each row showed significant difference at $p \leq 0.05$

3.3. Morphological characteristics

Plants grown in FYM, FYM + NPK and FYM + N amended soil showed significant decrease in root length than those grown under control and N amended soil. Shoot length was significantly higher in FYM amended soil **[Table–3]**. Due to presence of higher concentration of nutrients in FYM amended soil, the root did not go deeper into the soil in search of nutrients and hence its length remained shorter. Number of leaves and leaf area were also significantly higher in FYM treated plants compared to other amendments and control soil. Mgbeze and Abu [22] have found higher shoot height, number of leaves and leaf area of African yam bean (*Sphenostylis stenocarpa*) grown in FYM amended river sand. Among all the amendments, shoot biomass was highest in FYM amended soil followed by FYM + NPK, FYM + N, NPK, N and then in control soil. Root biomass, however, did not vary significantly among different amendments. Yield of plant was highest in soil amended with FYM **[Table–3]**. Singh et al. [31] have also found higher yield of wheat on FYM amended soil compared to those grown in only NPK amended soil. This may be because of slow release of nutrients under FYM amendment. The plants showed higher yield in FYM + NPK and FYM + N amended soil than NPK amended soil because of residual effects of FYM. Similar to the results of the present study, the yield of wheat crop was found to be enhanced by 43 % and 71 % in N + FYM and NPK + FYM treatments, respectively, over NPK treatment [32].

3.4. Physiological characteristics and pigment concentrations

Photosynthetic rate (Ps) and stomatal conductance (g_s) increased maximally by 14 and 6 %, respectively in plants grown in FYM amended soil compared to the control **[Table–4]**.

Table: 4. Physiological characteristics of palak grown under various amendments at the time of harvest (Mean ± 1 SE)

Characteristics	C	FYM	NPK	N	FYM + NPK	FYM + N
Photosynthetic rate (μ mol CO_2 $m^{-1}s^{-1}$)	16.67^{b} ± 1.20	19.0^{a} ± 0.58	14.0^{c} ± 0.57	14.33^{b} ± 0.88	15.33^{b} ± 0.67	16.67^{b} ± 0.67
Stomatal conductance (m mol $m^{-2}s^{-1}$)	1.64^{ab} ± 0.14	1.74^{ab} ± 0.08	1.42^{b} ± 0.09	1.40^{b} ± 0.05	1.53^{ab} ± 0.03	1.45^{b} ± 0.02
Transpiration rate (mol $H_2Om^{-2}s^{-1}$)	11.33^{a} ± 0.88	12.00^{a} ± 0.58	10.0^{b} ± 1.00	10.0^{b} ± 0.58	11.67^{a} ± 0.33	12.0^{a} ± 0.58
Water use efficiency	1.49^{a} ± 0.17	1.59^{a} ± 0.10	1.42^{a} ± 0.08	1.43^{a} ± 0.09	1.32^{a} ± 0.08	1.39^{a} ± 0.08
Fo (mv)	368.7^{b} ± 34.6	412.0^{a} ± 13.89	317.0^{c} ± 19.0	344.67^{b} ± 26.39	451.67^{a} ± 44.09	406.67^{a} ± 11.3
Fv (mv)	906.3^{b} ± 90.3	1053.0^{ab} ± 38.7	1146.7 ± 11.97	715.30^{b} ± 83.34	892.33^{a} ± 83.33	1015.7^{a} ± 61.3
Fm (mv)	1107^{b} ± 106.	1190.0^{ab} ± 61.4	1275.3 ± 37.35	935.0^{b} ± 28.87	1035.0^{b} ± 76.38	1015.7^{ab} ± 61.3
Fv/Fm	0.81^{a} ± 0.07	0.88^{a} ± 0.07	0.69^{b} ± 0.02	0.76^{a} ± 0.01	0.86^{a} ± 0.02	0.75^{b} ± 0.05

Different letters in each row showed significant difference at $p \leq 0.05$

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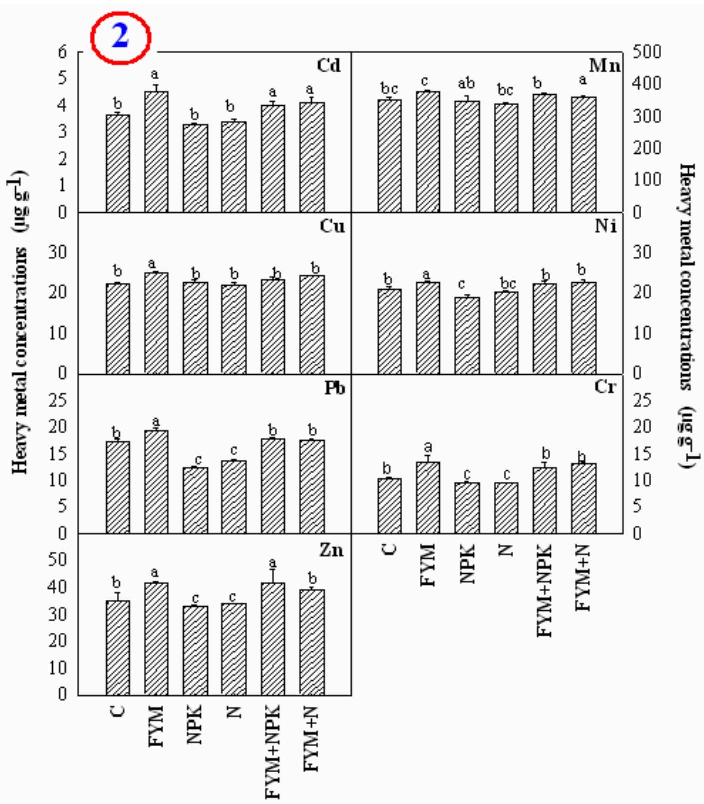
Due to presence of higher concentration of heavy metals under N and NPK amendment soil, plants showed reduction in transpiration rate compared to other amendments and control soil. Fo, Fv and Fm showed highest values in plants grown in FYM amended soil. The ratio of Fv/Fm ranges from 0.78 to 0.85 under healthy and unstressed condition in the plants [33]. In the present study, Fv/Fm ratio varied from 0.69 to 0.88 among different amendments with the lowest in NPK amended soil **[Table–4]**. Lower value of Fv/Fm in NPK amended soil showed stress condition in the plant. Chlorophyll a and b contents were highest in plants grown in FYM amended soil **[Figure–3]**. Plants grown in soil amended with combination of organic and inorganic fertilizers also showed increments in chlorophyll content compared to those where inorganic fertilizer was applied alone. Bokhtiar and Katsutoshi [34] have also found higher chlorophyll content in sugarcane plant grown in soil amended with organic fertilizer along with the inorganic fertilizer compared with inorganic fertilizer alone. Carotenoid content was highest in plants grown in NPK amended soil as compared to other amendments and control plants **[Figure–3]**. Carotenoids protect the plants by scavenging the free radicals generated under heavy metal stress. Although there was higher concentration of carotenoids in NPK amended soil, but it was not able to ameliorate the negative effect caused by excess heavy metal absorption in plants.



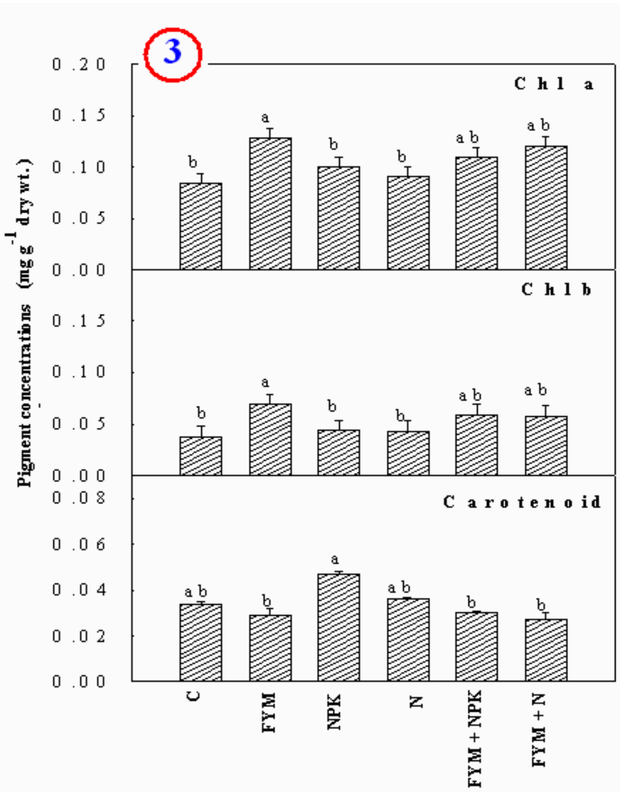


Fig: 2. Total heavy metal concentrations in the soil at the time of final sampling. Fig: 3. Pigment concentrations in palak at 40 DAG. In both the figures, values are mean ± 1 SE. Bars with different letters in each group showed significant difference at $p \leq 0.05$.

3.5. Biochemical characteristics

Palak plants grown in NPK amended soil showed maximum peroxidase activity and proline, ascorbic acid, phenol and thiol contents compared to the control **[Table–5]**. Antioxidants protect the plants by maintaining the balance between synthesis of free radicals and its detoxification [35]. Plants grown in NPK amended soil showed higher antioxidant levels, due to much higher availability of heavy metals compared to other treatments.

Heavy metal stress resulted into various harmful effects such as membrane damage that resulted into higher lipid peroxidation measured as MDA content in plants grown in NPK amended soil. Control plants also showed higher lipid peroxidation activity due to high heavy metal accumulation. Increase in lipid peroxidation under heavy metal stress is due to the generation of free radicals that distort the membrane architecture causing an oxidative damage [36].

Table: 5. Selected biochemical characteristics of palak grown under different amendments at harvesting time (Mean ± 1 SE)

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Characteristics	C	FYM	NPK	N	FYM + NPK	FYM + N
LPO (nmol ml^{-1} fresh wt.)	$0.73^{a} \pm 0.01$	$0.22^{c} \pm 0.02$	$0.72^{a} \pm 0.14$	$0.60^{b} \pm 0.41$	$0.41^{c} \pm 0.01$	$0.35^{c} \pm 0.02$
Peroxidase activity (µm purpurogallin min^{-1} g^{-1} fresh wt.)	$7.90^{a} \pm 0.51$	$5.17^{b} \pm 0.14$	$8.81^{a} \pm 1.65$	$5.18^{b} \pm 0.37$	$8.40^{a} \pm 0.51$	$5.43^{b} \pm 0.43$
Proline (mg g^{-1} fresh wt.)	$0.28^{a} \pm 0.03$	$0.22^{b} \pm 0.01$	$0.29^{a} \pm 0.03$	$0.26^{a} \pm 0.02$	$0.23^{b} \pm 0.02$	$0.18^{b} \pm 0.01$
Phenol (mg g^{-1} fresh wt.)	$5.78^{ab} \pm 0.74$	$4.38^{b} \pm 0.63$	$6.14^{a} \pm 0.49$	$5.88^{ab} \pm 0.46$	$4.96^{ab} \pm 0.44$	$4.31^{b} \pm 0.28$
Ascorbic acid (mg g^{-1} fresh wt.)	$0.052^{c} \pm 0.01$	$0.015^{d} \pm 0.01$	$0.104^{a} \pm 0.01$	$0.069^{bc} \pm 0.01$	$0.055^{c} \pm 0.01$	$0.079^{b} \pm 0.01$
Thiol (µmol g^{-1} fresh wt.)	$5.54^{b} \pm 0.36$	$3.73^{c} \pm 0.39$	$8.73^{a} \pm 0.97$	$4.02^{bc} \pm 0.17$	$3.02^{c} \pm 0.07$	$4.35^{bc} \pm 0.37$

Different letters in each row showed significant difference at $p \leq 0.05$

Plants grown in FYM amended soil showed percent reductions of 69 ,35, 21, 24, 71 and 33 in MDA content, peroxidase activity, proline, phenol, ascorbic acid and thiol contents, respectively as compared to the plants grown in the control soil. Due to lower availability of heavy metals in plants grown under FYM amended soil, the requirement of antioxidants was low and hence induction of antioxidants were also lower compared to other amendments and the control.

3.6. Heavy metal concentrations in plant parts

In both root and shoot portions, of concentrations of metals were minimum in FYM amended soil **[Figure–4a, –4b]**. Plants grown in FYM amended soil showed reductions of 42, 34, 17, 17, 33 and 41 % for Cd, Cu, Pb, Zn, Ni and Cr, respectively in shoot portion compared to the plants grown in the control soil. Singh et al. [28, 30] have also reported reductions in heavy metals in the plant parts under FYM amendment of contaminated soil. Plants grown in FYM+ NPK and FYM + N amended soil showed less concentrations of heavy metals compared to plants grown in NPK and N amended soil because the phyto availability of heavy metals was lower in the soil under combined treatment **[Figure – 4a, –4b]**. The residual effect of FYM along with the inorganic fertilizer is responsible for decreasing the concentrations of heavy metals in the plants.

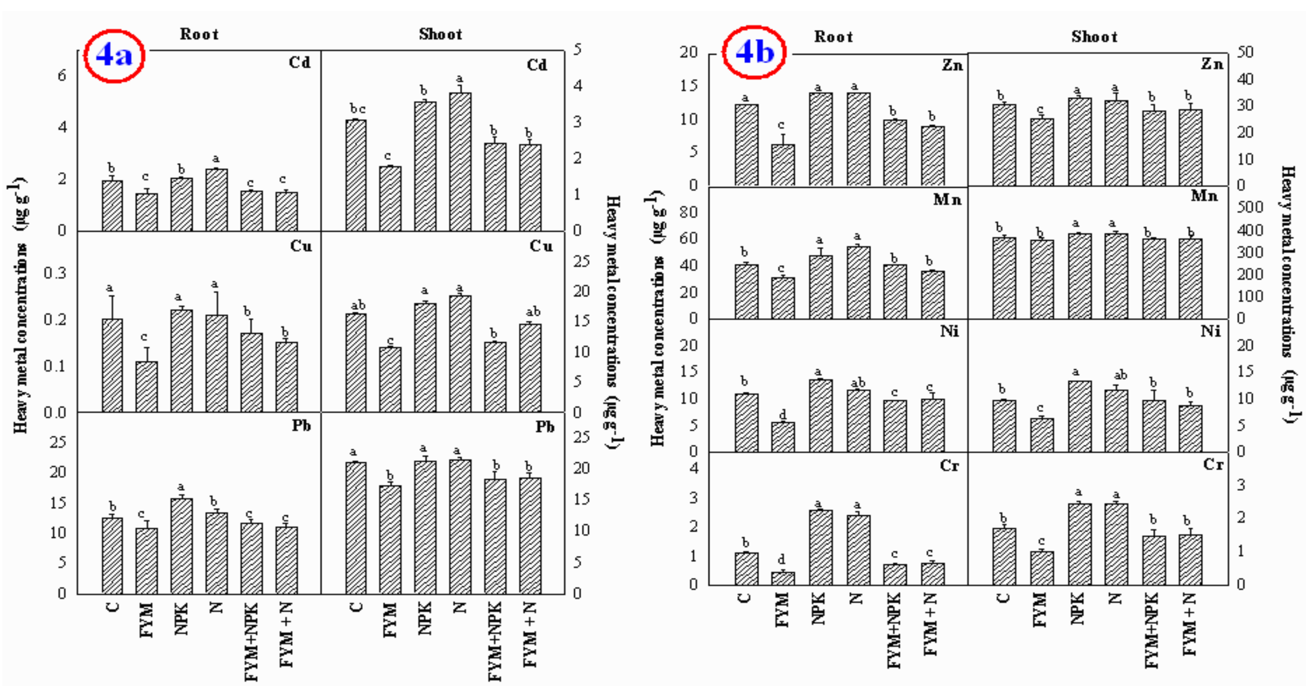

Fig: 4. (a). Cd, Cu and Pb concentrations in root and shoot portions of palak at the time of harvest. (b). Zn, Mn, Ni and Cr concentrations in root and shoot portions of palak at the time of harvest. Values are mean ± 1 SE. Bars with different letters in each group showed significant difference at p ≤ 0.05.

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[IV] CONCLUSIONS

Results of the present study conclude that among all the amendments, availability of heavy metals was higher in NPK and N amended soil and lower in soil amended with FYM alone and in FYM + NPK and FYM + N. Higher accumulation of heavy metals under NPK and N amendments led higher induction of antioxidants in the plants. Plants grown in FYM alone and FYM in combination with NPK and N showed better growth compared to other amendments and the control. The present study suggests that FYM alone and in combination with N and NPK (inorganic fertilizer) may be used to reduce the phytoavailability of heavy metals in the soil and consequently to maintain the physiological vitality and to improve the growth and yield of the plants at contaminated sites utilized for agriculture and horticulture purposes.

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REVIEW ARTICLE

ENERGY, ENVIRONMENT, AND SUSTAINABLE DEVELOPMENT IN SUDAN

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ABSTRACT

Application of new and renewable sources of energy available in Sudan is now a major issue in the future energy strategic planning for the alternative to the fossil conventional energy to provide part of the local energy demand. Like many of the African leaders in renewable energy utilisation, Sudan has a well-defined commitment to continue research, development, and implementation of new technologies. Sustainable low-carbon energy scenarios for the new century emphasise the untapped potential of renewable resources are needed. Rural areas of Sudan can benefit from this transition. The increased availability of reliable and efficient energy services stimulates new development alternatives. It is concluded that renewable environmentally friendly energy must be encouraged, promoted, implemented, and demonstrated by full-scale plant especially for use in remote rural areas.

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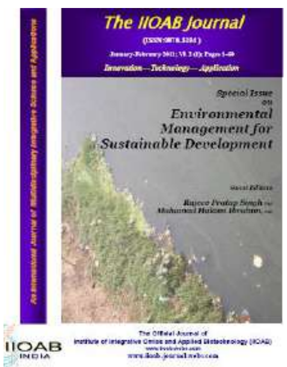

Keywords: ***Sudan; energy; impacts on environment; sustainable development***

[I] INTRODUCTION

This section is an introduction to the energy problem and the possible savings that can be achieved through improving energy performance and the use of solar energy sources. The relevance and importance of the study is discussed in the section, which, also, highlights the objectives of the study, and the scope of the article. Energy issues affect every aspect of modern society. These issues have been of primary concern, since the second oil crisis and the Gulf War. Energy problems are associated with distribution, access and security of supply. Particularly for the energy-deficient countries and remote islands/areas, renewable energy appears to be sustainable and a clean source of energy derived from nature [1]. The utilisation of available renewable energy sources like solar, wind and biomass energy is of practical importance for future socio-economic development of the country. Sudan is an agricultural country with fertile land, plenty of water resources, livestock, forestry resources and agricultural residues. Energy is one of the key factors for the development of national economies in the Sudan. Energy sources are divided into two main types; conventional energy (woody biomass, petroleum products and electricity); and non-conventional energy (solar, wind, hydro, etc.). Sudan possesses a relatively high abundance of sunshine, solar radiation, moderate wind speeds, hydro and biomass energy resources. Application of the new and renewable sources of energy available in the Sudan is a major issue in the future strategic planning for an alternative to the fossil conventional energy to provide part of the local energy demand. Sudan is an important case study in the context of renewable energy. It has a long history of meeting its energy needs through renewables. Sudan's renewables portfolio is broad and diverse, due in part to the country's wide range of climates and landscapes. Like many of the African leaders in renewable energy utilisation, Sudan has a well-defined commitment to continue research, development and implementation of new technologies.

As for example, ''climate change is a hot issue in world politics''. The use of fossil fuel is seen as a cause of critical global warming. Long-term energy options currently considered include the petroleum, electricity, and biomass. Sustainability is increasingly becoming an element of world politics, although there is not yet agreement on a clear definition and indicators are still not yet fully agreed upon that would effectively enable the establishment of the sustainable development, which is so eagerly sought. However, the snowball is rolling: the process of designing sustainable development has started, and it is only a matter of political will, negotiations and time for it to accelerate, hopefully in the right direction. The job is tough, and the variety of stakeholders involved in today's globalisation process makes the whole story even more interesting and challenging. And yet, globalisation and sustainable development are bound to become tautological. The goal of sustainability has different meanings and measures specific to various regions of the planet, each with

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their own economics, histories, and cultures. A pathway to sustainable development that is reasonable, achievable and hence natural in one country. Growing concerns about social and environmental sustainability have led to increased interest in planning for the energy utility sector because of its large resource requirements and production of emissions. A number of conflicting trends combine to make the energy sector a major concern, even though a clear definition of how to measure progress toward sustainability is lacking. These trends include imminent competition in the electricity industry, global climate change, expected long-term growth in population and pressure to balance living standards (including per capital energy consumption). Designing and implementing a sustainable energy sector will be a key element of defining and creating a sustainable society. In the electricity industry, the question of strategic planning for sustainability seems to conflict with the shorter time horizons associated with market forces as deregulation replaces vertical integration.

Sudan is an agricultural country with fertile land, plenty of water resources, livestock, forestry resources, and agricultural residues. Energy is one of the key factors for the development of national economies in Sudan. An overview of the energy situation in Sudan is introduced with reference to the end uses and regional distribution. Energy sources are divided into two main types; conventional energy (woody biomass, petroleum products, and electricity); and non-conventional energy (solar, wind, hydro, etc.). Sudan enjoys a relatively high abundance of sunshine, solar radiation, moderate wind speeds, hydropower, and biomass energy resources. Sudan is an important case study in the context of renewable energy. It has a long history of meeting its energy needs through renewables. Sudan's renewables portfolio is broad and diverse, due in part to the country's wide range of climates and landscapes **[Appendixes-1 and -2]**.

In developed countries, most investments in electricity generation have paid back their initial capital costs. Research and development of new electricity generation technologies are well under way, and these technologies hold good promise of achieving commercial feasibility. New investments in electricity generation have not been aggressively pursued in recent years. Rather, policies for existing energy infrastructures have included improved options for more sustainable electricity generation, e.g., less pollution, higher fuel efficiency and life extension. However, are, at best, only interim solutions. Stakeholders interaction is essential to create a culture of sustainability, with educational, regulatory, economic, environmental and ethical dimensions that can have an impact on society overall. Such a culture of sustainability must be designed, managed and measured in ways compatible with societal attitudes towards risk, including changes in perspective over medium and long-term time horizons. Risk-related concepts play a large part in what technologies the public views as sustainable, whether it concerns potentially large accidents from electric generation technologies, greenhouse gas emissions, vulnerability to natural disasters, or decommissioning and site reclamation problems. Research and development costs and expected lead times related to electricity generation, also play a role in stakeholder positions and public attitudes [2].

There are increasing concerns but the sustainability of the energy sector, ranging from impacts of current operation to the choice of future options for system development. These concerns include such issues as health and safety, environmental emissions, use of energy and materials resources, regulated versus competitive markets, vulnerability of electrical energy networks, cost and equity issues among users of diverse size, and appropriate technology for the development and commercialisation of improved supply and end-use equipment. Such concerns span the geographic spectrum from local issues such as cost and siting, to regional issues like acid rain [3], state and national issues such as deregulation and social acceptance of competing technologies, and global issues such as climate change [4]. Decision-makers and planners in the energy sector must address climate change and global warming issues, and fit them into a broader framework of national and world energy policies. The methodological framework needed to assist decision-makers can be generalised in part, because most of these problems share common elements, and can be characterised by their combination of: (1) Complexity (2) dispersed solutions (3) finite resources, and (4) societal impacts.

The move towards a de-carbonised world, driven partly by climate science and partly by the business opportunities it offers, will need the promotion of environmentally friendly alternatives, if an acceptable stabilisation level of atmospheric carbon dioxide is to be achieved. This requires the harnessing and use of natural resources that produce no air pollution or greenhouse gases and provides comfortable coexistence of human, livestock, and plants. This study reviews the energy-using technologies based on natural resources, which are available to and applicable in the farming industry. Globally, buildings are responsible for approximately 40% of the total world annual energy consumption. Most of this energy is for the provision of lighting, heating, cooling, and air conditioning. Increasing awareness of the environmental impact of CO2 and NOx and CFCs emissions triggered a renewed interest in environmentally friendly cooling, and heating technologies. Under the 1997 Montreal Protocol, governments agreed to phase out chemicals used as refrigerants that have the potential to destroy stratospheric ozone. It was therefore considered desirable to reduce energy consumption and decrease the rate of depletion of world energy reserves and pollution of the environment. In the areas of power systems plan, one of the most pressing problems those exemplifiers this combination of characteristics is the intersection between:

- The rapid liberalisation of energy industries driving short-term actions to maximise stakeholder values, and
- The possible restrictions on greenhouse gas emissions proposed to meet the problem of global climate change.

National Electricity Corporation (NEC) had the goal of improving electricity system planning methodological with

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regard to sustainability. Within this broad aim, this search has specific goals, including:

- To involve a wide-range of electricity sector participants, including utilities, regulators, environmentalists, and customers.
- To identify and implement a wider range of measures that will be more direct indicators of sustainability than the current response impacts and pollutant outputs.
- To create improved electricity sector modeling tools that simulate electricity system operation under competition, and include transmission and distribution effects more directly into strategic planning.
- To consider technology options beyond the current next generation.
- To increase the comprehensiveness of current life-cycle assessment, by including broader range of technologies, inputs and outputs are sensitive to technology choice versus the state of underlying production and transport infrastructures.
- To improve decision analysis tools to assist stakeholders in reaching consensus on complex alternatives, option portfolios, and flexible contingencies.
- To make the data and models developed for understanding the relationship between power systems and sustainability, available and usable through Internet access.

NEC handled wide variety of electricity generation technologies [Table-1], including:

Thermal (fossil, combined cycle, combustion turbines).
Storage (pumped hydro, batteries, compressed air).
Non-dispatchable technologies (solar, wind, cogeneration, load management).

The data required to perform the trade-off analysis simulation can be classified according to the divisions given in [Table-2] the overall system or individual plants, and the existing situation or future development.

Table: 1. Comparison between different energy consumed in Sudan (GWh) [5]

Year	Electricity	Petroleum	Biomass	Renewable Technologies
1975	800	550	3000	50
1980	900	600	3500	60
1985	1200	650	4000	100
1990	1300	700	6000	120
1995	1400	800	7000	150
2000	1500	900	8000	200

1.1. Geographic Profile of the Sudan

This section comprises a comprehensive review of geographical location of the Sudan, energy sources, the environment and sustainable development. It includes the renewable energy technologies, energy efficiency systems, energy conservation scenarios, energy savings and other mitigation measures necessary to reduce climate change. Sudan is the largest country of the African continent, with an area of approximately one million square miles (2.5 x 10^6 km^2). Sudan is a federal republic located in eastern Africa. It extends between latitudes 3º'N and 23º'N, and longitudes 21º 45'E and 39º'E. Sudan is a relatively sparsely populated country. The total population according to the 2009 census was 39 x 10^6 inhabitants. The growth rate is 2.8%/y, and population density is 14 persons per square kilometres. The country is divided into 26 states and a federal district, in which the capital, Khartoum is located. Sudan is known as a country of plentiful water, rich in land, with the highest total and renewable supply of fresh water in the region (eastern Africa). Sudan is considered one of the least developed countries, with a per capita income of less than US $ 400 and a real growth rate of 0.2 % of real gross domestic product (GDP) during the last ten years. However, during 1980s the real growth rate of GDP was negative mainly due to drought and desertification. The backbone of Sudan's economy is its agricultural sector. The agricultural sector determines to a great extent the economic performance of the Sudanese economy. In fact the country can be rescued by proper organisation and utilisation of its agricultural potential. Recent development due to rehabilitation and improvement in the agricultural sector has raised the share to 41% [6].

Table: 2. Classifications of data requirements [7]

	Plant data	System data
Existing data	Size Life Cost (fixed and var Operation and Maintenance) Forced outage Maintenance Efficiency Fuel Emissions	Peak load Load shape Capital costs Fuel costs Depreciation Rate of return Taxes
Future data	All of above, plus Capital costs Construction trajectory Date in service	System lead growth Fuel price growth Fuel import limits Inflation

1.2. Energy Situation in Sudan

Among the renewable energy sources, biomass seems one of the most interesting because of its share of the total energy consumption of the Sudan is high at 87% and the techniques for converting it to useful energy are not necessarily sophisticated. Implementation of biomass-based energy programmes will not, of course, be a definitive solution to the country's energy problem, but it will bring new insight for efficient energy use in the household sector, especially in rural areas where more than 70% of the populations live (25 million). The estimates are based on the recoverable energy potential from the main agricultural residues, livestock farming wastes, forestry and wood processing residues; and municipal wastes.

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Fuelwood, animal wastes, agricultural crop residues and logging wastes have been used through direct burning in the Sudan for many years. These sources are often called non-commercial energy sources, but in the Sudan fuelwood is a tradable commodity since it is the primary fuel of rural areas and the urban poor section. Traditional fuels predominate in rural areas; almost all biomass energy is consumed in the household sector for heating, cleaning and cooking needs of rural people. Especially in the villages (households on the high plateau) the preparation of three stone fires is very attractive to the villagers. In this method, food and plant residues are put in a large boiler with water and cooked on a traditional stove at the outside the house for animal feed, because cooked food and plant residues are cheaper than flour and bran. Nevertheless, this method consumes much more fuelwood than the cooking on the stoves method. On the other hand, wood is the most practical fuel for serving a large number of people because the size of the batch of food is only limited by the volume of the pot and not by the size of the stove's burner. Fuelwood is also convenient for cooking the meal of meat as a cutlet meatball and meat roasted on a revolving vertical spit.

Special attention should therefore be given to reviewing forest resources, plantation programmes and the possibilities of substitution of fuelwood for commercial fuels or for other fuels such as biogas. The main sources of fuelwood supply in the country can be broadly be grouped into two main categories, i.e., forest sources (forests under the control of forest departments) and non-forest sources (private farmland and wild lands). Women, assisted by children almost always, perform the gathering of fuelwood in rural areas of developing countries. As fuelwood becomes scarce, which is the case in many parts of the world, the collection time has increased and although men do not perceive it, this has many undesirable consequences, which can be clearly seen in many rural region of the Sudan. Women have less time for their other important functions, such as cooking, washing, water collection and child rearing which may affect the nutrition and health of the entire family. Wood energy is, for many countries, one of the few locally available sources of energy, which they can afford. Its substitution by imported fossil fuels, as has often been carelessly recommended, should attentively be evaluated to avoid undesirable political, economic and social consequences. This will also contribute to the amelioration of environmental conditions by replacing conventional fuels with renewable energies that produce no air pollution or greenhouse gases. Renewable energy needs, especially in rural areas and small communities. The role of renewable is big in solving essential life problems especially in rural areas for people and their resources development like the availing of energy for the medical services for people and animal, provision of water, education, communication and rural small industries.

[II] ENVIRONMENTAL ASPECTS

Environmental pollution is a major problem facing all nations of the world. People have caused air pollution (since they learned proceeding to use fire), but man-made air pollution anthropogenic air pollution) has rapidly increased since industrialisation began. Many volatile organic compounds and trace metals are emitted into the atmosphere by human activities. The pollutants emitted into the atmosphere do not remain confined to the area near the source of emission or to the local environment, and can be transported over long distances, and create regional and global environmental problems.

A great challenge facing the global community today is to make the industrial economy more like the biosphere, that is, to make it a more closed system. This would save energy, reduce waste and pollution, and reduce costs. In short, it would enhance sustainability. Often, it is technically feasible to recycle waste in one of several different ways. For some wastes there are powerful arguments for incineration with energy recovery, rather than material recycling. Cleaner production approach and pollution control measures are needed in the recycling sector as much as in others. The industrial sector world widely is responsible for about one third of anthropogenic emissions of carbon dioxide, the most important greenhouse gas. Industry is also an important emitter of several other greenhouse gases. And many of industry's products emit greenhouse gases as well, either during use or after they become waste. Opportunities exist for substantial reducing industrial emissions through more efficient production and use of energy. Fuel substitutions, the use of alternative energy technologies, process modification, and by revising materials strategies to make use of less energy and greenhouse gas intensive materials. Industry has an additional role to play through the design of products that use less energy and materials and produce lower greenhouse gas emissions.

Development in the environmental sense is a rather recent concern relating to the need to manage scarce natural resources in a prudent manner-because human welfare ultimately depends on ecological services. The environmental interpretation of sustainability focuses on the overall viability and health of ecological systems- defined in terms of a comprehensive, multiscale, dynamic, hierarchical measure of resilience, vigour and organisation. Natural resource degradation, pollution and loss of biodiversity are detrimental because they increase vulnerability, undermine system health, and reduce resilience. The environmental issues include:

- Global and transnational (climate change, ozone layer depletion).
- Natural habitats (forests and other ecosystems).
- Land (agricultural zones).
- Water resources (river basin, aquifer, water shed).
- Urban-industrial (metropolitan area, air-shed).

Environmental sustainability depends on several factors, including:

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- Climate change (magnitude and frequency of shocks).
- Systems vulnerability (extent of impact damage).
- System resilience (ability to recover from impacts).

Economic importance of environmental issue is increasing, and new technologies are expected to reduce pollution derived both from productive processes and products, with costs that are still unknown. This is due to market uncertainty, weak appropriability regime, lack of a dominant design, and difficulties in reconfiguring organisational routines. The degradation of the global environment is one of the most serious energy issues. Various options are proposed and investigated to mitigate climate change, acid rain or other environmental problems. Additionally, the following aspects play a fundamental role in developing environmental technologies, pointing out how technological trajectories depend both on exogenous market conditions and endogenous firm competencies:

(1) Regulations concerning introduction of Zero Emission Vehicles (ZEV), create market demand and business development for new technologies.
(2) Each stage of technology development requires alternative forms of division and coordination of innovative labour, upstream and downstream industries are involved in new forms of inter-firm relationships, causing a reconfiguration of product architectures and reducing effects of path dependency.
(3) Product differentiation increases firm capabilities to plan at the same time technology reduction and customer selection, while meeting requirements concerning network externalities.
(4) It is necessary to find and/or create alternative funding sources for each research, development and design stage of the new technologies.

The Sudan energy consumption is modest by international standards. The energy balance is dominated by biomass. Woody biomass makes up 71 percent, petroleum products 19%, non-woody biomass 8% and hydropower 2% [8]. The total Consumption of the country is as low as 6.3 million tons of oil equivalent (TOE) while the total primary supply is about 11 million TOES. This means that 43% of the energy is lost in converting wood to charcoal. Distances transported nearly always exceed 600 km. The major consumers are households (78% of the total energy). The largest consumers of firewood are brick markers. The efficiency of the traditional charcoal stoves is as low as 16 percent. The forest cover of Sudan receded from 25% in 1956 to 12% in 2001. Sudan is a member of the club of the 18 countries of "Least Forest Covered". Oil fields are in Addar Yale, Bashair and Heglig. Crude from the former two is transported by river barges and trucked respectively.

The environmental impacts of the various processes involved have never been addressed. Further more some of the crude is wastefully used directly as a source of energy. The contribution of the two fields to the GNP has never been disclosed neither have their negative impacts on the environment. The oil is pumped from the various wells and fields through a system of 160 km of pipes the central facility at Heglig. The crude is de-watered at Heglig and then piped 1610 km to the Red Sea export terminal. The infrastructure of the operation includes an extensive network of raised roads and five field facilities. In addition to the central facility at Heglig, there is the airport, the contractors' camp, the newly-founded towns of Heglig and Keilak Al Kharassan, five pumping stations, a metering station at El Obeid, two refineries at El Obeid and Khartoum and the large refinery al El Gaili. Oil is a principal factor in Sudanese politics. It is the government's main source of income and the oil sector is driving economic growth. Meanwhile, the oil industry is poorly managed and highly politicised. Rather than contributing to an environment of peace and equitable development, it remains a source of strife and division.

[III] SUSTAINABILITY

Absolute sustainability of electricity supply is a simple concept: no depletion of world resources and no ongoing accumulation of residues. Relative sustainability is a useful concept in comparing the sustainability of two or more generation technologies. Therefore, only renewables are absolutely sustainable, and nuclear is more sustainable than fossil. However, any discussion about sustainability must not neglect the ability or otherwise of the new technologies to support the satisfactory operation of the electricity supply infrastructure. The electricity supply system has been developed to have a high degree of resilience against the loss of transmission circuits and major generators, as well as unusually large and rapid load changes. It is unlikely that consumers would tolerate any reduction in the quality of the service, even if this were the result of the adoption of otherwise benign generation technologies. Renewables are generally weather-dependent and as such their likely output can be predicted but not controlled. The only control possible is to reduce the output below that available from the resource at any given time. Therefore, to safeguard system stability and security, renewables must be used in conjunction with other, controllable, generation and with large-scale energy storage. There is a substantial cost associated with this provision.

It is useful to codify all aspects of sustainability, thus ensuring that all factors are taken into account for each and every development proposal. Therefore, with the intention of promoting debate, a sustainability matrix is presented **[Table-3]**. The following considerations are proposed:

(1) Long-term availability of the energy source or fuel.
(2) Price stability of energy source or fuel.
(3) Acceptability or otherwise of by-products of the generation process.
(4) Grid services, particularly controllability of real and reactive power output.
(5) Technological stability, likelihood of rapid technical obsolescence.
(6) Knowledge base of applying the technology.
(7) Life of the installation – a dam may last more than 100 years, but a gas turbine probably will not.
(8) Maintenance requirement of the plant.

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This article envisages the ways of integrated development of combined heat and power sector in Sudan. However, the assumptions and objectives seem to be applicable to other

Table: 3. Sustainability matrixes [9]

Power categories	1*	2*	3*	4*	5*	6*	7*	8*	9*	Index
Conventional coal fired stream plant	3	1	1	5	1	1	4	3	3	22
Oil fired stream plant	2	1	1	5	3	3	4	3	3	25
Combined cycle gas turbine	2	3	2	4	4	4	4	2	4	29
Micro combined heat and power	2	3	2	4	4	4	3	2	4	29
Nuclear	4	4	3	5	4	4	3	2	3	32
Hydropower	5	5	5	3	5	5	5	4	2	39
Tidal power	5	5	5	2	5	5	5	4	2	38
Onshore wind	5	5	5	2	5	5	4	4	3	38
Offshore wind	5	5	5	2	5	5	3	4	4	38
Land-fill gases	3	5	3	1	3	4	4	3	2	28
Municipal incineration	5	5	4	3	4	4	4	3	4	36
Biomass, field and forest crops plus waste straw	5	5	4	3	4	4	4	3	4	36
Import	1	1	5	1	5	5	5	5	5	33
Hydro pumped storage	-	-	5	5	5	5	5	5	2	32
Electrochemical storage	-	-	4	4	4	4	4	4	5	29
Diesel	2	1	1	1	4	5	3	4	4	25

1* fuel availability, 2* price stability of fuel, 3* by-product acceptability, 4* grid services, 5* technological obsolescence, 6* knowledge base, 7* life of the installation, 8* maintenance requirement, 9* infrastructure requirements.

Table: 4: Effective biomass resource utilisation

Subject	Tools	Constraints
Utilisation and land clearance for agriculture expansion	• Stumpage fees • Control • Extension • Conversion • Technology	• Policy • Fuel-wood planning • Lack of extension • Institutional
Utilisation of agricultural residues	• Briquetting • Carbonisation • Carbonisation and briquetting • Fermentation • Gasification	• Capital • Pricing • Policy and legislation • Social acceptability

Table: 5. Agricultural residues routes for development

Source	Process	Product	End use
Agricultural residues	Direct Processing Processing Carbonisation Fermentation	Combustion Briquettes Carbonisation (small-scale) Briquettes Carbonised Biogas	Rural poor Urban household Industrial use Industrial use Limited household use Rural household (self sufficiency) Urban fuel Energy services Household Industry
Agricultural, and animal residues	Direct Briquettes Carbonisation Carbonisation Fermentation	Combustion Direct combustion Carbonised Briquettes Biogas	(Save or less efficiency as wood) (Similar end use devices or improved) Use Briquettes use Use

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developing countries having plenty of agricultural, forest and animal resources. The process of biomass power generation would certainly reduce dependence on imported fossil fuel and provides a clear indication in reducing the GHG emissions in the environment and it could claim carbon credit more effectively. Most of the heat is produced by large CHP plants (gas-fired combined cycle plants using natural gas, biomass, waste or biogas). DH is energy efficient because of the way the heat is produced and the required temperature level is an important factor. Buildings can be heated to temperature of 21^oC and domestic hot water (DHW) can be supplied with a temperature of 55^oC using energy sources that are most efficient when producing low temperature levels (<95^oC) for the DH water. Most of these heat sources are CO_2 neutral or emit low levels. Only a few of these sources are available to small individual systems at a reasonably cost, whereas DH schemes because of the plant's size and location can have access to most of the heat sources and at a low cost. Low temperature DH, with return temperatures of around 30-40^oC can utilise the following heat sources:

- Efficient use of CHP by extracting heat at low calorific value (CV).
- Efficient use of biomass or gas boilers by condensing heat in economisers.
- Efficient utilisation of geothermal energy.
- Direct utilisation of excess low temperature heat from industrial processes.
- Efficient use of large-scale solar heating plants.

Heat tariffs may include a number of components such as: a connection charge, a fixed charge and a variable energy charge. Also, consumers may be incentivised to lower the return temperature. Hence, it is difficult to generalise but the heat practice for any DH company no matter what the ownership structure can be highlighted as follows:

- To develop and maintain a development plan for the connection of new consumers.
- To evaluate the options for least cost production of heat.
- To implement the most competitive solutions by signing agreements with other companies or by implementing own investment projects.
- To monitor all internal costs and with the help of benchmarking, improve the efficiency of the company.
- To maintain a good relationship with the consumer and deliver heat supply services at a sufficient quality.

Sustainable energy is energy that, in its production or consumption, has minimal negative impacts on human health and the healthy functioning of vital ecological systems, including the global environment. It is an accepted fact that renewable energy is a sustainable form of energy, which has attracted more attention during recent years. A great amount of renewable energy potential, environmental interest, as well as economic consideration of fossil fuel consumption and high emphasis of sustainable development for the future will be needed. Explanations for the use of inefficient agricultural-environmental polices include: the high cost of information required to measure benefits on a site-specific basis, information asymmetries between government agencies and farm decision makers that result in high implementation costs, distribution effects and political considerations [10]. Achieving the aim of agric-environment schemes through:

- Sustain the beauty and diversity of the landscape.
- Improve and extend wildlife habitats.
- Conserve archaeological sites and historic features.
- Improve opportunities for countryside enjoyment.
- Restore neglected land or features, and create new habitats and landscapes.

The data required to perform the trade-off analysis simulation can be classified according to the divisions given in **[Table-4]** the overall system or individual plants, and the existing situation or future development. The effective economic utilisations of these resources are shown in **[Table-5]**, but their use is hindered by many problems such as those related to harvesting, collection, and transportation, besides the photo-sanitary control regulations. Biomass energy is experiencing a surge in interest stemming from a combination of factors, e.g., greater recognition of its current role and future potential contribution as a modern fuel, global environmental benefits, its development and entrepreneurial opportunities, etc. Possible routes of biomass energy development are shown in Table 11. Biomass resources should be divided into residues or dedicated resources, the latter including firewood and charcoal can also be produced from forest residues **[Table-6]**.

Table: 6. Biomass residues and current use

Type of residue	Current use / availability
Wood industry waste	No residues available
Vegetable crop residues	Animal feed
Food processing residue	Energy needs
Sorghum, millet, wheat residues	Fodder, and building materials
Groundnut shells	Fodder, brick making, direct fining oil mills
Cotton stalks	Domestic fuel considerable amounts available for short period
Sugar, bagasse, molasses	Fodder, energy need, ethanol production (surplus available)
Manure	Fertilizer, brick making, plastering (*Zibala*)

Biomass energy includes fuelwood, agricultural residues, animal wastes, charcoal and other fuels derived from biological sources. It currently accounts for about 14% of world energy consumption. Biomass is the main source of energy for many developed and developing countries. In Sudan, energy wood is available in the form of forest chips, fuelwood, wood waste, wood pellets and it is also produced to a very limited extent from willow crops in short rotation forestry. The major part of wood

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harvested in the forest area approximately 108 million hectares ends up as energy wood directly or indirectly after having been used for other purposes first. In 2000, the biomass share of the total energy consumption of the country was 87%. The main advantages are related to energy, agriculture and environment problems, are foreseeable both at regional level and at worldwide level and can be summarised as follows:

- Development of new know-how and production of technological innovation.
- Reduction of dependence on import of energy and related products.

Even with modest assumptions about the availability of land, comprehensive fuel-wood farming programmes offer significant energy, economic and environmental benefits. These benefits would be dispersed in rural areas where they are greatly needed and can serve as linkages for further rural economic development. The nations, as a whole would benefit from savings in foreign exchange, improved energy security, and socio-economic improvements. With a nine-fold increase in forest – plantation cover, the nation's resource base would be greatly improved. The international community would benefit from pollution reduction, climate mitigation, and the increased trading opportunities that arise from new income sources. The aim of any modern biomass energy systems must be:

- To maximise yields with minimum inputs.
- Utilisation and selection of adequate plant materials and processes.
- Optimum use of land, water, and fertiliser.
- Create an adequate infrastructure and strong R and D base.
- Reduction of environmental impact of energy production (greenhouse effect, air pollution, waste degradation).
- Demand, by rational energy use, by recovering heat and the use of more green energies.

This study was a step towards achieving that goal. The adoption of green or sustainable approaches to the way in which society is run is seen as an important strategy in finding a solution to the energy problem. The key factors to reducing and controlling CO_2, which is the major contributor to global warming, are the use of alternative approaches to energy generation and the

[IV] ENVIRONMENTAL POLICIES AND INDUSTRIAL COMPETITIVES

The industrial development strategy in Sudan gives priority to the rehabilitation of the major industrial areas with respect to improvement of infrastructure such as roads, water supply, power supply, sewer systems and other factors. This strategy also takes into consideration the importance of incorporating the

- Substitution of food crops and reduction of food surpluses and of related economic burdens.
- Utilisation of marginal lands and of set aside lands and reduction of related socio-economic and environmental problems (soil erosion, urbanisation, landscape deterioration, etc.).

exploration of how these alternatives are used today and may be used in the future as green energy sources.

Furthermore, investigating the potential to make use of more and more of its waste. Household waste, vegetable market waste, and waste from the cotton stalks, leather, and pulp; and paper industries can be used to produce useful energy either by direct incineration, gasification, digestion (biogas production), fermentation, or cogeneration.

Therefore, effort has to be made to reduce fossil energy use and to promote green energies, particularly in the building sector. Energy use reductions can be achieved by minimising the energy

Even with modest assumptions about the availability of land, comprehensive fuel-wood farming programmes offer significant energy, economic and environmental benefits. These benefits would be dispersed in rural areas where they are greatly needed and can serve as linkages for further rural economic development. The nations as a whole would benefit from savings in foreign exchange, improved energy security, and socio-economic improvements. With a nine-fold increase in forest – plantation cover, a nation's resource base would be greatly improved. The international community would benefit from pollution reduction, climate mitigation, and the increased trading opportunities that arise from new income sources.

There is a huge availability of biomass energy resources in Sudan. These resources are scatterly distributed all over the country. In western Sudan, there are briquetting of groundnut shells plants in operation at Nyala and El Obeid. In central Sudan, briquetting plants of cotton stalk installed at Wad El Shafie. In southern Sudan, biogas plants in operation using water hyacinth. Sugarcane bagasse and sugarcane trash already provide a significant amount of biomass for electricity generation. It is known that sugarcane is a perennial crop and sugarcane bagasse available in a particular period of the year. The bagasse-gasifier plants in Kenana are used as standby or at the peak times or if there is any problem in main grid. environmental dimension into economic development plans. However, the relationship between environmental policies and industrial competitiveness has not been adequately examined. For the near future, the real issue concerns the effectiveness of environmental expenditures in terms of reduction of pollution emissions per unit of output. A number of issues relevant to this central concern are presented as follows:

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4.1. Implementing ecologically sustainable industrial development strategies

The United Nations Industrial Development Organisation (UNIDO) Agenda 21, (1997), Vienna for achieving sustainable development in the 21st century calls on governments to adopt National Strategies (NS) for sustainable development that ‘‘build on and harmonise the various sectoral, social and environmental policies that are operating in the country’’ [4]. NS focuses almost exclusively on development issues and does not integrate industrial and environmental concerns. It does not consider industrial specific environmental objectives or time frames for achieving them. Moreover, it does not specify how specific industrial sub-sectors and plants will meet environmental objectives. Finally, it is formulated with minimal involvement of industrial institutions and private sector associations. To bring together industrial development and environmental objectives it is necessary to:

- Establish environmental goals and action plans for the industrial sector.
- Develop an appropriate mix of policy instruments that support the goals of those plans.
- Design appropriate monitoring and enforcement measurements to realise those goals.

4.2. Applying cleaner Production processes and techniques

Traditional approaches to pollution reduction have been based on the application of end of pipe technologies in order to meet discharge standards. However, the growing recognition that reduction at source is a potentially more cost effective method of abatement is resulting in replacing end of pipe technologies with cleaner production processes. Major constraints in adopting cleaner production methods relate to:

- Lack of awareness about the environmental and financial benefits of cleaner production activities.
- Lack of information about techniques and technologies.
- Inadequate financial resources to purchase imported technologies.

A coordinated effect by industry, government and international organisations can go a long way in overcoming these constraints. In this context key questions that need to be addressed are as follows:

(a) Need for local capacity building, information dissemination, training and education.
(b) Need for sub-sectoral demonstration projects.
(c) Need for increased cooperation with environmental market sectors in developed countries.
(d) Need for life cycle analysis and research on environmentally compatible products.

4.3. Implementing environmental management systems

Environmental management systems (EMSs) are necessary to enable plant to achieve and demonstrate sound environmental performance by controlling the environmental impact of their activities, products and services. The basic tools to ensure compliance with national and/or international requirements and continually improve its environmental performance include:

- Environmental auditing.
- Environmental reporting, and
- Environmental impact assessments.

In addition, the adoption of EMS may require extensive training of corporate staff. A practical and effective means of doing this is through the design and support of joint capacity strengthening programmes by industry association and bilateral and multilateral agencies.

4.4. Managing and conserving water resources

It is estimated that by year 2025, there will be a global crisis in water resources. Accelerated growth of industry will lead to increase in industrial water use. Moreover, major industrial water pollutant load is expected to increase considerably in the near future. Therefore, to better manage water resources by industry, there is a real need for integrating demand trend and use patterns. The main elements of an industrial management strategy can be identified as follows:

- Analytical services.
- Promotional services.
- Services for the development of industry and water supply infrastructure.

4.5. Using market based instruments (MBIs) to internalise environmental costs

As complements to command and control measures for resource conservation and pollution prevention in industry. MBIs represent a useful and efficient cost effective policy measures that internalise environmental costs. A plant’s decision to invest in clean production depends primarily on the following factors:

(a) Relative costs of pollution control in overall production costs.
(b) Price elasticities of supply and demand for intermediary and final goods, and
(c) Competitive position of plant in a particular industrial sector.

4.6.Counteracting threats from eco-labelling requirements

The increasing export orientation of production makes it necessary to maintain competitive position in world markets. The emergence of a wide variety of eco-labelling requirements and

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lack of timely information on multitude of scheme may adversely affect certain export sectors. Needed initiatives to counteracting perceived threats could be presented as follows:

- Information dissemination.
- Life cycle analysis.
- Establishing certification centres.
- Infrastructure support.

4.7.Implementing the United Nations (UN) framework convention on climate change

The UN climate change convention entered into force on 21st March 1994. The convention objective is the stabilisation of greenhouse gas concentration in the atmosphere at safe levels. For industry, responding to this convention will undoubtedly be a major challenge. Industry will be directly affected. Sudan as party to this convention is obliged to take a number of actions and cooperates effectively in order to meet this challenge. Sudan has to contribute to the common goal of reducing greenhouse gases emissions by taking precautionary measures to mitigate causes and anticipate impacts of climate change. However, there may not be adequate means to do so, and Sudan will therefore require international assistance. The main requirements are:

- Access to best energy-efficient technologies available on the world market, where such technologies are relevant to our natural resources endowments, our industrial requirements and are cost effective.
- Building an energy-efficient capital stock by accelerating the development of low energy intensity processes and equipment.
- Strengthening national capabilities for energy-efficient design and manufacturing.

Areas where technical expertise to implement the convention is necessary include:

- Preparing national communications on greenhouse gas emissions. The communications are supported to contain an assessment of the magnitudes and sources of greenhouse gases as well as identification of reduction methods.
- Supporting technology transfer for improvement in the efficiency of fuel based power generation.
- Promotion technology transfer for the use of renewable sources of energy such as biomass, wind, solar, hydro, etc.
- Developing and implementing technology transfer for energy efficiency programmes in industry, in complementarities with cleaner production/pollution prevention measures.
- Analysing the impact of climate change response measures on the economic and industrial development of the country, with the view to identifying economically viable technology options for reducing greenhouse gas emissions from the production and consumption of energy.

4.8.Addressing concerns of small and medium scale industry (SMI)

Small and medium scale enterprises not only contribute to productivity growth and employment but are also important as collective sources of localised pollution loading such as organic wastes in water effluent, as well as hazardous wastes, heavy metal sludge, solvents, waste oils, acidic and alkaline wastes, photo wastes, etc. Often, these wastes are disposed of in unsafe manure and are extremely difficult to monitor. The cost of control in relation to output is too high, so even a modest increase in the costs (of environmental regulations) may threaten prevention and control may be well known and easily available, there is no guarantee that they will be adopted. Moreover, even when policy measures are in place, their enforcement and monitoring is a real problem for SMI sector on account of their large numbers and diversity. It is clear that environment problems of SMIs require special attention and special measures to address their particular problems.

[V] ENERGY AND ENVIRONMENT

5.1. Petroleum industry pollution and greenhouse gases emissions in Sudan

The activities of oil exploration in Sudan began in late 1950s in the coastal areas of Red Sea. The results of exploration indicated that there is considerable amount of natural and liquefied gases in Suwakin and Bashair, and the quantities were estimated between 45-326 × 10^9 cubic meters [12]. According to the increasing oil industry activities in Sudan such as production, refining and export/consumption, and if we consider the entire fuel cycle, namely: exploration, extraction, preparation/transformation, transportation, storage, pollution, including the increase in greenhouse gases, as result of petroleum industry will be very significant in the forthcoming future. Exploitation has started in the south of Sudan and exports have begun recently. In the year 2005 about 2 × 10^9 tonnes of petroleum products were burnt in Sudan [11]. This amount will be doubled in the year 2010. There is a shortage of information concerning the area of greenhouse gases recording in Sudan.

5.2. Privatisation and price liberalisation in energy source supplies

The privatisation and price liberalisation in energy fields has to some secured (but not fully). Availability and adequate energy supplies to the major productive sectors. The result is that, the present situation of energy supplies is for better than ten years ago. The investment law has also encourage the participation of the investors from the national level as well as from the international friendly and sisters' countries to invest in energy sources supply such as:

- Petroleum products (import in particular) in the northern states.
- Electricity generation (in some states) through providing large diesel engine units.

The readily implementation of electricity price liberalisation has some extent release the National Electricity Corporation (NEC) from the heavy dependency of government subsidies, and a noticeable improved of NEC management, and electricity supplies are achieved.

5.3. *Synthesis of the renewable energy*

Although the overall impact of renewables has been unnecessary low, the experience has clearly demonstrated their potential as sustainable energy alternatives. There has been substantial learning in disseminating and managing various technologies on account of:

- Scale: with increasing numbers, teething problems have been overcome and better knowledge has been gained in different aspects related to planning, implementation, operation and maintenance.
- Indigenisation: through joint ventures with international industry, the technology transfer process has been facilitated, helping in developing local production capacities.
- Infrastructure: a strong infrastructure has been created over the years to provide the technical, operational and managerial support to intervention programmes. This includes research institutions, training agencies, NGOs, financial intermediaries, *etc.*
- Diverse strategies: though the whole renewable energy programme started with the same technology push approach, diversification occurred over a period of time in terms of strategies and to promote different technologies according to market conditions [Table-7].

5.4. *Geothermal energy*

The use of geothermal energy in Sudan has the following special characteristics:

Disadvantages are geothermal energy is a low-temperature resource tied to specific locations and can be used only in specific types of system and minerals in some thermal waters can produce scaling problems that increase costs. Advantages:

- Sudan has a big geothermal energy potential and geothermal energy can be environmentally sound if wastewater or brine is properly disposed of (e.g., by reinjection) and there is no air pollution.
- The dependency on imported energy sources could be decreased.
- The number of jobs can be increased.
- Competitiveness of geothermal energy should increase owing to projected increases in domestic prices of competing fossil fuels.
- Geothermal wells do not give low temperature heat; certainly they give much higher than solar flat plate collectors.

Not only does cost of the fuel drive the researcher to explore an alternative source of energy, but ozone layer problems are other factor that should be considered in searching for cheap and clean energy sources [Table-8]. Solar energy is an unlimited, cheap and clean source of energy, which has been utilised to replace conventional energy, but there still is a need to develop a technology to utilise the solar energy. Nowadays, solar energy is widely used in heating water, dehumidifying air and generating electrical energy for many domestic, agricultural and industrial applications. For these applications, the most important piece of the system equipment is the solar collector in which solar energy is converted into heat or electrical power. Although there are several designs for solar energy collecting devices, flat-plate collectors are the most common and popular type. They have the advantages of being simple to build, employing locally available materials, they are easy to operate and maintain, they have the ability to function even during cloudy or hazy days with diffuse solar radiation only, and they are capable of integration into a roof or a wall structure.

Fresh water supply is one of the most limiting conditions for the populations of arid regions. Following methods can solve the problem of providing arid areas with fresh water:

(1) Transportation of water from other locations.
(2) Desalination of saline water (ground and underground).
(3) Extraction of water from atmospheric air.

Transportation of water through these regions is usually very expensive, and desalination depends on the presence of saline water resources, which are usually rare in arid regions. The extraction of water from atmospheric air can be accomplished by two methods: cooling moist air, and absorbing water vapour from moist air using a solid or a liquid desiccant, with subsequent recovery for the extracted water by heating the desiccant and condensing the evaporated water. The significant increase in recent years of the number of rural electrification systems, using photovoltaic technology for illumination or water pumping in order to reduce the burden of capital costs per unit of generated power.

The power generation and supply for isolated areas can be effectively achieved using wind energy as the principle energy source. The savings are not only economic but also environmentally sound. However, the transient nature of the energy supply that is delivered to the power system from wind-turbine poses significant challenges to the design and control of the system. The efficiency of such systems can only improve with more effective control and a better understanding of the characteristics of these types of systems.

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Table: 7. Diversity of promotion strategies [12]

Technology	Strategy	Key change agents	Mechanisms
Power generation			
Wind	Government-enabled market pull	Private sector State utilities Manufacturers	Fiscal incentives Multilateral finance
Small-hydro	Government-enabled market pull	Private sector State utilities Manufacturers	Fiscal incentives Multilateral finance
Biomass cogeneration	Government-enabled market pull	Private sector Government	Fiscal incentives Low interest finance
Solar PV	Combination of demonstration and pull	NGOs Intermediaries Manufacturers	Flexible loan serving Maintenance by intermediaries
Thermal energy			
Biogas	Technology push	Government NGOs Turnkey workers	Cash subsidy Training support
Improved stoves	Technology push	Government NGOs Self-employed workers	Cash subsidy Training support
Solar cookers	Push in the beginning Currently market pull	Government Manufacturers	Mini-subsidy Segmentation with focus On small towns and cities
Solar water heaters	Push in the beginning Currently market pull	Government Manufacturers	Segmentation with focus on industrial systems

Considering energy crises and pollution problems today, investigations have been concentrated on decreasing fuel consumption by using alternative fuels and lowering the concentration of toxic components in combustion products. Hydrogen is considered as a renewable clean energy source. Two methods using hydrogen in spark ignition engines were investigated in Sudan. The first method is to mix hydrogen with gasoline. The second concerns using hydrogen as a standard fuel. The advantage of the hydrogen-supplemented fuel is that it requires a smaller quantity of hydrogen, which considerably reduces the problems connected with hydrogen storage in the automobile. A hydrogen-gasoline fuelled engine generally develops lower maximum power and higher NO_x emission compared to an equivalent gasoline engine. Hydrogen can be used as a supplementary fuel in modern spark ignition engines without major changes and it can help save a considerable part of the available oil and save our environment from toxic pollutants.

Depletion of fossil fuels and fluctuating prices has rekindled an interest in the development of renewable fuels/energy sources such as biomass. There is a critical need for development of alternative, appropriate, affordable methods of cooking for use in Sudan. Population pressures on remaining forest resources have resulted in shortages of fuel available for household use and yielded adverse environmental effects. Solar cooker use has the potential to alleviate both problems by reducing dependence on wood as a cooking fuel. However, to gain acceptance and motivate use, there is a need to develop many different solar cooker designs. Each design needs to be suited to specific climates, customers and economic factors. The development requires a good fundamental understanding of the relationship between key design variables and performance. The most commonly used type of solar cooker is the box type. Box type cookers depend on heat retention. They are slow to heat up, but work well even where there is diffuse radiation, convective heat loss caused by wind, intermittent cloud cover, and low ambient temperatures. Despite the variation in approach flat plate, concentrating, and box type) and large variety of inventions and products, the total potential design space is largely unexplored. Systematic examination of the design space requires either extensive testing or models with broad predictive capacity.

The availability of methane and/or natural gas has lead to a worldwide spread of internal combustion engines running on the dual fuel concept. Gaseous fuels also promise to be suitable for higher compression engine

Since it is known that they resist knock more than conventional liquid fuels, as well as producing less polluting exhaust gases, if appropriate conditions are satisfied for its mixing and combustion. Therefore, it is more economical and of environmental advantage to use natural gases in diesel engines, which use the dual fuel concept.

5.5. Climate change, global warming and the enhanced greenhouse effect

Changes in the global climate (mean temperature, precipitation, etc.) could also threaten stability of a range of critical interlinked physical, ecological and social systems and subsystems [13]. An important social principle is that the climate change should not be allowed to worsen existing inequities- although climate

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change policy cannot be expected to address all prevailing equity issues. Some special aspects include:

- The establishment of an equitable and participative global framework for making and implementing collective decisions about climate change.
- Reducing the potential for social disruption and conflicts arising from climate change impacts.
- Protection of threatened cultures and preservation of cultural diversity.

Climate change is one of the most serious concerns of our society. The gases causing the greenhouse effect are carbon dioxide, methane, nitrous oxide, chlorofluorocarbons, troposphere ozone, and stratospheric water vapour. Carbon dioxide is an important greenhouse gas and a major agent of climate change and the most significant greenhouse gas contributing about half of the total greenhouse effect [14]. The recent concentration of atmospheric CO_2 is 25% higher than the pre-industrial level [15]. This increase is primarily due to fossil fuel combustion and deforestation.

Table: 8. Correlation of solar radiation with other weather parameters in Sudan (Yearly averages) [16]

Station	Mean temp. (ºC)	Sunshine duration (h)	Solar radiation (MJm^{-2} day^{-1})	Wind velocity (ms^{-1})
Port Sudan	28.4	9.0	20.87	5.1
Shambat	29.7	9.9	22.82	4.5
Wadi Medani	28.4	9.8	22.84	4.5
El Fasher	25.8	9.6	22.80	3.4
Abu Na'ama	28.2	8.8	21.90	3.1
Ghazala Gawazat	27.2	9.3	21.72	3.0
Malakal	27.9	7.8	19.90	2.8
Juba	27.6	7.8	19.59	1.5
Dongola	27.2	10.5	24.06	4.6
Toker	28.8	7.3	17.60	4.1
Hudeiba	29.3	10.0	22.37	4.0
Aroma	29.1	9.6	21.40	4.2
El Showak	26.3	9.7	22.90	4.1
Zalingei	24.5	8.8	22.98	2.7
Babanusa	28.2	8.9	21.73	2.8
Kadugli	27.5	8.5	21.30	2.7

Climate change might pose a serious threat to our ecological and socio-economic systems, unless measures are investigated to mitigate the rising accumulation of atmospheric CO_2. Green-space in urban ecosystems can reduce atmospheric C levels in three ways. Here, green-space is defined as soil surface area capable of supporting vegetation and the vegetation being supported. First, urban trees and shrubs directly sequester and accumulate atmospheric C in the process of their growth through photosynthesis. Second, urban vegetation decreases building cooling demand by shading and evapotranspiration, and heating demand by wind speed reduction, thereby reducing C emissions associated with fossil fuel use. Third, urban soils store organic C from litter-fall, until it is returned to the atmosphere by decomposition. On the other hand, buildings, factories, and automobiles in urban landscapes release C through fossil fuel consumption.

Industry's use of fossil fuels has been blamed for our warming climate, when coal, gas and oil are burnt, they release harmful gases, which trap heat in the atmosphere and cause global warming. However, there has been an ongoing debate on this subject, as scientists have struggled to distinguish between changes, which are human induced, and those, which could be put down to natural climate variability. Industrialised countries have the highest emission levels, and must shoulder the greatest responsibility for global warming. But action must also be taken by developing countries to avoid future increases in emission levels as their economies develop and population grows. Human activities that emit carbon dioxide (CO_2), the most significant contributor to potential climate change, occur primarily from fossil fuel production. Consequently, efforts to control CO_2 emissions could have serious, negative consequences for economic growth, employment, investment, trade and the standard living of individuals everywhere. Scientifically, it is difficult to predict the relation between global temperature and greenhouse gas concentrations. The climate system contains many processes that will change if warming occurs. Critical processes include heat transfer by winds and currents, the hydrological cycle involving evaporation, precipitation, runoff and groundwater, and the formation of clouds, snow, and ice, all of which display enormous natural variability. The equipment and infrastructure for energy supply and use are designed with long lifetimes, and the premature turnover of capital stock involves significant costs. Economic benefits occur if capital stock is replaced with more efficient equipment in step with its normal replacement cycle, and if opportunities to reduce future emissions are taken in the world they should be less costly. Such flexible approaches would allow society to take account of evolving scientific and technological knowledge, and to gain experience in designing policies to address climate change. Focusing only on CO_2 emissions neglects the full richness of the environmental data, but has been done for several reasons:

- CO_2 is an important global environmental indicator.
- CO_2 emissions can show very significant increases, as Sudan currently produces very little CO_2 from the electricity sector.
- CO_2 and other air pollutants are linked together to new generation capacity; So CO_2 serves as a surrogate for most other emissions.

The environmental characteristics of products have become increasingly important to consumers [17-18]. Firms have responded by placing eco-labels on products that highlight the item's environmental attributes and by introducing new, or redesigned green products [19]. Governments and non-governmental organisations have also responded by organising, implementing, and verifying eco-labelling programmes that

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cover thousands of products, which international efforts to standardise environmental labelling schemes have also emerged. From a policy perspective, one aim of eco-labels is to educate consumers about the environmental impacts of the product's manufacture, use, and disposal, thereby leading to a change in purchasing behaviour and ultimately, to a reduction in negative impacts. Further, eco-labelling policies may promote environmental objectives without production site command and control methods and are seen a way of meeting global environmental objectives while complying with international trade agreements. Recent techniques for economically valuing environmental impacts:

- Effect on production.
- Effect on health.
- Defensive or preventive costs.
- Replacement cost and shadow projects.
- Travel cost.
- Property value.
- Wage differences (the wage differential method attempts to relate changes in the wage rate to environmental conditions, after accounting for the effects of all factors other than environment (e.g., age, skill level, job responsibility, etc.) that might influence wages).

5.6. Major energy consuming sectors

Sudan is still considered as one of the 25 most developing African countries. Agriculture is the backbone of economic and social development in Sudan. About 80% of the population depends on agriculture, and all other sectors are largely dependent on it. Agriculture contributes to about 41% of the gross national product (GNP) and 95% of all earnings. Agriculture determines for the last 30 years the degree of performance growth of the national economy.

5.6.1. Agriculture sector

During the last decades agriculture contributed by about 41% to the Sudan GNP. This share remained stable until 1984-1985 when Sudan was seriously hit by drought and desertification, which led to food shortages, deforestation, and also, by socio-economic effects caused the imposed civil war. The result dropped the agriculture share to about 37%. Recent development due rehabilitation and improvement in agricultural sector in 1994 has raised the share to 41%. This share was reflected in providing raw materials to local industries and an increased export earning besides raising percentage of employment among population. This sector consumed 2.5% of the total energy consumption (28% from electricity, 14.8% from fossil fuels, and the rest from biomass fuels) [20].

5.6.1. Industrial sector

The industrial sector is mainly suffering from power shortages, which is the prime mover to the large, medium and small industries. The industrial sector was consuming 5.7% of the total energy consumption, distributed as follows: 55% from petroleum products, 13% from biomass and 32% from electricity [20].

5.6.3. Domestic use

Household is the major energy consumer. It consumed 92% of the total biomass consumption in form of firewood and charcoal. From electricity this sector consumed 60% of the total consumption, and 5.5% of petroleum products.

5.6.4. Transport sector

The transportation sector (railways, ships, boats, etc.) was not being efficient for the last two decades because of serious damage happened to its infrastructure (roads, workshops, and maintenance centres, etc.). It consumed 10% of the total energy consumption and utilized 60% of the total petroleum products supplied.

Sudan is an energy importing country and the energy requirements has been supplied through imports that have caused financial problems. Because of the economical problems in Sudan today, the Sudanese energy policy should be concentrated on assurance of energy supply, reliability, domestic sufficiency, in time, in economic terms, and renewability. Therefore as a renewable energy source, biomass (especially fuelwood) seems interesting because its share of the total energy production at 87% is high and the techniques for converting it to useful energy are easy. On the other hand, biomass may, however, see greatly expanded use in response to the environmental problems caused by fossil fuel use in the country. Biomass has been proposed to have a central role to play in future, more sustainable energy scenarios. For this to become a reality several real problems need to be overcome. In Sudan as in other developing countries modernisation of biomass energy provision is an urgent necessity for the sake of human health, protection of the environment, and climate change abatement. Given sufficient recognition, resources and research biomass could become the environmentally friendly fuel of the future.

[VI] DISCUSSION

There is strong scientific evidence that the average temperature of the earth's surface is rising. This was a result of the increased concentration of carbon dioxide (CO_2), and other greenhouse gases (GHGs) in the atmosphere as released by burning fossil fuels [21]. This global warming will eventually lead to substantial changes in the world's climate, which will, in turn, have a major impact on human life and the environment [22]. Energy use reductions can be achieved by minimising the energy demand, by rational energy use, by recovering heat and the use of more green energies. This study was a step towards achieving this goal. The adoption of green or sustainable approaches to the way in which society is run is seen as an important strategy in finding a solution to the energy problem. The key factors to reducing and controlling CO_2, which is the major contributor to global warming, are the use of alternative approaches to energy generation and the exploration of how these alternatives are used today and may be used in the future as green energy sources. Even with modest assumptions about the availability of land, comprehensive fuel-wood farming programmes offer significant energy, economic and environmental benefits. These benefits would be dispersed in rural areas where they are greatly needed and can serve as linkages for further rural economic development. The nations as a whole would benefit from savings in foreign exchange, improved energy security, and socio-economic improvements. With a nine-fold increase in forest – plantation cover, the nation's resource base would be greatly improved. The international community would benefit from pollution reduction, climate mitigation, and the increased trading opportunities that arise from new income sources. The non-technical issues, which have recently gained attention, include: (1) Environmental and ecological factors e.g., carbon sequestration, reforestation and revegetation. (2) Renewables as a CO_2 neutral replacement for fossil fuels. (3) Greater recognition of the importance of renewable energy, particularly modern biomass energy carriers, at the policy and planning levels. (4) Greater recognition of the difficulties of gathering good and reliable biomass energy data, and efforts to improve it. (5) Studies on the detrimental health efforts of biomass energy particularly from traditional energy users. This study discusses a comprehensive review of biomass energy sources, environment and sustainable development. This includes all the biomass energy technologies, energy efficiency systems, energy conservation scenarios, energy savings and other mitigation measures necessary to reduce climate change.

Energy is an essential factor in development since it stimulates, and supports economic growth; and development. Fossil fuels, especially oil and natural gas, are finite in extent, and should be regarded as depleting assets, and efforts are oriented to search for new sources of energy. The clamour all over the world for the need to conserve energy and the environment has intensified as traditional energy resources continue to dwindle whilst the environment becomes increasingly degraded. Biomass energy supply in Sudan contributed 87% of total energy supply since 1980's. The basic form of biomass comes mainly from firewood, charcoal and crop residues. Out of total fuel wood and charcoal supplies 92% was consumed in household sector with most of firewood consumption in the rural areas [23]. Alternatively energy sources can potentially help fulfill the acute energy demand and sustain economic growth in many regions of the world. Bioenergy is beginning to gain importance in the global fight to prevent climate change. The scope for exploiting organic waste as a source of energy is not limited to direct incineration or burning refuse-derived fuels. Biogas, biofuels and woody biomass are other forms of energy sources that can be derived from organic waste materials. These renewable energy sources have significant potential in the fight against climate change.

There is an unmistakable link between energy and sustainable human development. Energy is not an end in itself, but an essential tool to facilitate social and economic activities. Thus, the lack of available energy services correlates closely with many challenges of sustainable development, such as poverty alleviation, the advancement of women, protection of the environment, and jobs creation. Emphasis on institution-building and enhanced policy dialogue is necessary to create the social, economic, and politically enabling conditions for a transition to a more sustainable future. On the other hand, biomass energy technologies are a promising option, with a potentially large impact for Sudan as with other developing countries, where the current levels of energy services are low. Biomass accounts for about one third of all energy in developing countries as a whole, and nearly 96% in some of least developed countries. The convention on Biological Diversity set conservation of biodiversity on the world agenda. Gaps in knowledge need to be addressed for actions to be effective and sustainable. Gaps include: species diversity, microorganisms and their ecological roles, ecological and geographical status of species, human capacity to access and forecast bio-ecological degradation. Requirements for global inventories call for worldwide collaboration. Criteria for setting priorities need to be formulated and agreed. Global inventorying needs a collaborative international effort, perhaps under the aegis of the Convention on Biological Diversity. The recently formulated global taxonomy initiatives are a step in the right direction.

This article envisages the ways of integrated development of combined heat and power sector in Sudan. However, the assumptions and objectives seem to be applicable to other developing countries having plenty of agricultural, forest and animal resources. The process of biomass power generation would certainly reduce dependence on imported fossil fuel and provides a clear indication in reducing the GHG emissions in the environment and it could claim carbon credit more effectively. Most of the heat is produced by large CHP plants (gas-fired combined cycle plants using natural gas, biomass, waste or biogas). DH is energy efficient because of the way the heat is produced and the required temperature level is an important factor. Buildings can be heated to temperature of 21^{o}C and domestic hot water (DHW) can be supplied with a temperature of 55^{o}C using energy sources that are most efficient when producing low temperature levels (<95^{o}C) for the DH water. Most of these heat sources are CO_2 neutral or emit low levels. Only a few of these sources are available to small individual systems at a reasonably cost, whereas DH schemes because of the plant's size and location can have access to most of the heat sources and at a low cost. Low temperature DH, with return temperatures of around 30-40^{o}C can utilise the following heat sources:

- Efficient use of CHP by extracting heat at low calorific value (CV).
- Efficient use of biomass or gas boilers by condensing heat in economisers.
- Efficient utilisation of geothermal energy.
- Direct utilisation of excess low temperature heat from industrial processes.
- Efficient use of large-scale solar heating plants.

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Heat tariffs may include a number of components such as: a connection charge, a fixed charge and a variable energy charge. Also, consumers may be incentivised to lower the return temperature. Hence, it is difficult to generalise but the heat practice for any DH company no matter what the ownership structure can be highlighted as follows:

- To develop and maintain a development plan for the connection of new consumers.
- To evaluate the options for least cost production of heat.
- To implement the most competitive solutions by signing agreements with other companies or by implementing own investment projects.
- To monitor all internal costs and with the help of benchmarking, improve the efficiency of the company.
- To maintain a good relationship with the consumer and deliver heat supply services at a sufficient quality.

Sustainable energy is energy that, in its production or consumption, has minimal negative impacts on human health and the healthy functioning of vital ecological systems, including the global environment. It is an accepted fact that renewable energy is a sustainable form of energy, which has attracted more attention during recent years. A great amount of renewable energy potential, environmental interest, as well as economic consideration of fossil fuel consumption and high emphasis of sustainable development for the future will be needed. Explanations for the use of inefficient agricultural-environmental polices include: the high cost of information required to measure benefits on a site-specific basis, information asymmetries between government agencies and farm decision makers that result in high implementation costs, distribution effects and political considerations [24]. Achieving the aim of agric-environment schemes through:

- Sustain the beauty and diversity of the landscape.
- Improve and extend wildlife habitats.
- Conserve archaeological sites and historic features.
- Improve opportunities for countryside enjoyment.
- Restore neglected land or features, and create new habitats and landscapes.

The key factors to reducing and controlling CO_2, which is the major contributor to global warming, are the use of alternative approaches to energy generation and the exploration of how these alternatives are used today and may be used in the future as green energy sources. Even with modest assumptions about the availability of land, comprehensive fuel-wood farming programmes offer significant energy, economic and environmental benefits. These benefits would be dispersed in rural areas where they are greatly needed and can serve as linkages for further rural economic development. The nations as a whole would benefit from savings in foreign exchange, improved energy security, and socio-economic improvements. With a nine-fold increase in forest – plantation cover, a nation's resource base would be greatly improved. The international community would benefit from pollution reduction, climate mitigation, and the increased trading opportunities that arise from new income sources.

There is a huge availability of biomass energy resources in Sudan. These resources are scatterly distributed all over the country. In western Sudan, there are briquetting of groundnut shells plants in operation at Nyala and El Obeid. In central Sudan, briquetting plants of cotton stalk installed at Wad El Shafie. In southern Sudan, biogas plants in operation using water hyacinth. Sugarcane bagasse and sugarcane trash already provide a significant amount of biomass for electricity generation. It is known that sugarcane is a perennial crop and sugarcane bagasse available in a particular period of the year. The bagasse-gasifier plants in Kenana are used as standby or at the peak times or if there is any problem in main grid.

Special attention should therefore be given to reviewing forest resources, plantation programmes and the possibilities of substitution of fuelwood for commercial fuels or for other fuels such as biogas. The main sources of fuelwood supply in the country can be broadly be grouped into two main categories, i.e., forest sources (forests under the control of forest departments) and non-forest sources (private farmland and wild lands). Women, assisted by children almost always, perform the gathering of fuelwood in rural areas of developing countries. As fuelwood becomes scarce, which is the case in many parts of the world, the collection time has increased and although men do not perceive it, this has many undesirable consequences, which can be clearly seen in many rural region of Sudan. Women have less time for their other important functions, such as cooking, washing, water collection and child rearing which may affect the nutrition and health of the entire family. Wood energy is, for many countries, one of the few locally available sources of energy, which they can afford. Its substitution by imported fossil fuels, as has often been carelessly recommended, should attentively be evaluated to avoid undesirable political, economic and social consequences. This will also contribute to the amelioration of environmental conditions by replacing conventional fuels with renewable energies that produce no air pollution or greenhouse gases. Renewable energy is needed, especially in rural areas and small communities. The role of renewable is big in solving essential life problems especially in rural areas for people and their resources development like the availing of energy for the medical services for people and animal, provision of water, education, communication and rural small industries.

Mitigation measures that could be under taken to influence the effect of oil industry and use that may contribute in decreasing greenhouse gases (GHGs) emissions and decelerate the threat of global climate change may include the following:

- Controlling GHGs emissions by improving the efficiency of energy use, changing equipment and operating procedures.

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- Controlling GHGs emission detection techniques in oil production, transportation and refining processes in Sudan.
- More efficient use of energy-intensive materials and changes in consumption patterns.
- A shift to low carbon fuels, especially in designing new refineries.
- The development of alternative energy sources (e.g., biomass, solar, wind, hydro-electrical and cogeneration).
- The development of effective environment standards, policies, laws and regulations particularly in the field of oil industry.
- Activating and supporting environmental and pollution control activities within the Ministry of Energy and Mining (MEM) to effectively cope with the evolving oil industry in Sudan.

6.1. Oil production

Oil was discovered in Sudan in the mid-1970s, but production did not start until 1999. The pioneer companies Chevron and Shell were forced to bow out in 1984, after the outbreak of civil war. They eventually sold their rights in 1990, booking a $1 billion loss. Oil in Sudan accounts for 92.6% of the country's export revenues and with most of its producing oilfields located in the South of the country, the management of the oil industry is a key factor that will determine the future of the country. The oil industry is poorly supervised and highly politicised, and as such, rather than contributing to an enabling environment for peace and equitable development, a source of strife and division **[Figure -1]**.

The focus for 2007 is on both exploration and development. The operators of the producing blocks are implementing aggressive exploration programmes **[Table-9]**. With the companies wanting to achieve payback as quickly as possible, development of discoveries is likely to be prompt.

Table: 9. Sudan's oil reserves [27]

Year	Proven reserves (bln bbl)	Oil production (10^3 bbl/d)
1981	0.2	0.0
1991	0.3	0.0
2001	0.7	211
2005	6.4	355
2006	6.4	397

Refineries:

- Khartoum (50/50 joint venture between the Sudanese government and the CNPC, capacity of (100,000 bbl/d).
- Port Sudan Refinery (21,700 bbl/d).
- Petronas has agreed to joint venture with the Sudanese government to build a new refinery in Port Sudan with capacity of (100,000 bbl/d) to treat Dar Blend crude; to be operational in 2010.
- The small top-up refinery in Abu Gabra is planned for closure 2010. There are plans to build a refinery in Kosti Sudan's oil production will probably in 2010, but revenues may be maintained for another ten years at current levels, depending on the development of oil prices and whether the Dar Blend refinery will indeed be a price booster.

Oil is a principal factor in Sudanese politics. It is the government's main source of income and the oil sector is driving economic growth. Meanwhile, the oil industry is poorly managed and highly politicised. Rather than contributing to an environment of peace and equitable development, it remains a source of strife and division [25].

The Sudanese oil industry is exceptionally profitable because oil companies are exempted from paying taxes in Sudan. These conditions may have been quite reasonable in 1997.

The main Sudanese oil contracts were negotiated in the 1990s, when oil was being traded for less than $20 per barrel and the Sudanese governments had to offer lucrative conditions to attract investments. It makes a big difference, however, whether the companies' share of 20% to 40% of the Profit Oil is sold at $20 or $60 per barrel. Oil is now traded at $90 per barrel and more, boosting profits for the companies and leaving the government of the Sudan with too small a share.

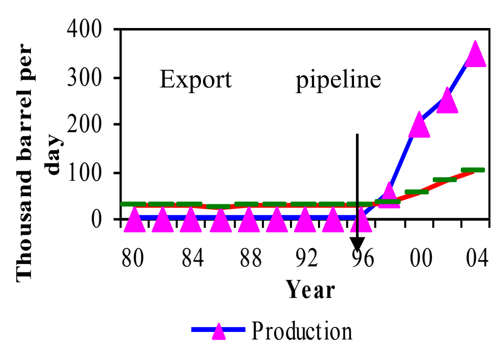

Fig: 1. Sudan's oil production and consumption 1980-2005 [26]

6.2. Sudan's experience in renewable energy technologies

In Sudan, great attention is given to the utilisation of the renewable, and the overall renewable energy potential of the country. Three distinct groups contribute to research, development and utilisation of the resources. These are:
1. Research institutes.
2. Universities, and
3. Private-sector.

Participation and roles in technology diffuse:

1. Government:
- Improved economic competitiveness of technology.
- Support information flow technical financial viability, and resource assessment.
- Support training.
- Undertake R&D.

2. NGOs:
- Training.
- Extension.
- Assessment of local needs.
- Demonstration.
- Promotion of small-scale production.

3. Private-Sector:
- Production.
- Assembly, maintenance and spare-parts supply.
- Marketing.

6.3. Policy development

The non-technical issues, which have recently gained attention include:

- Environmental and ecological factors e.g., carbon sequestration, reforestation and revegetation.
- Biomass as CO_2 neutral replacement for fossil fuels.
- Greater recognition of the importance of renewable energy, particularly modern biomass energy carriers, at the policy and planning levels.
- Greater recognition of the difficulties of gathering good and reliable renewable energy data, and efforts to improve it.
- Studies on the detrimental health efforts of renewable energy particularly from traditional energy users.
- Greater awareness of the need to internalise the externality cost of conventional energy carriers to place them on more equal terms with alternative energy sources.

6.4. The future

(1) In the most of the developing countries, the governments acknowledge that, renewable energy can resolve many pressing problems. Yet, the matter stops at this level ''Acknowledgement''. Much more is needed, like laws regulating and encouraging business, tax concessions, both to investors and customers, and most of all, a sustained, coordinated and well-planned official publicity campaign to enlight, inform and educate the public at a large.
(2) To avoid the problems of fuel altogether (uncertain availability and skyrocketing prices), and minimise spare-parts, solar and wind pumps are proposed to replace diesel engines in the predominant irrigation areas.
(3) Local manufacture, whenever possible, is to be emphasised to avail renewable energy devices since limited funds are the main constraints in commercialisation and dissemination of the technology. Low cost devices as well as reliable devices have to be provided.
(4) Embarking on conservation energy and reduction of pollution of environment to be undertaken without delay:

- To save on fossil fuel for premium users/export.
- To accelerate development of new and/or remote lands otherwise deprived of conventional energy sources.
- As a preventive measure against shortage of future energy supply against prospective national energy demand.

(5) Launching of public awareness campaigns among investor's particularly small-scale entrepreneurs and end users of renewable energy technologies to highlight the importance and benefits of renewables.
(6) To direct Sudan resources away from feeding wars and the arms industry towards real development, this will serve the noble ends of peace and progress.
(7) The energy crisis is a national issue and not only a concern of the energy sector, and the country has to learn to live with the crisis for a long period, and develop policies, institutions and manpower for longer term, more effective solutions.
(8) To invest in research and development through the existing specialised bodies e.g., Energy Research Institute (ERI).
(9) To encourage co-operation between nations, a fact this will be much easier in this era of information and the communications revolution.
(10) Government should give incentives to encourage the household sector to use renewable energy technologies instead of conventional energy.
(11) Promotion research and development, demonstration and adaptation of renewable energy resources (solar, wind, biomass, and mini-hydro, etc.) amongst national, regional, and international organisations which seek clean, safe, and abundant energy sources.
(12) Execute joint investments between the private sector and the financing entities to disseminate the renewables with technical support from the research and development entities.
(13) Promotion the general acceptance of renewable energy strategies by supporting comprehensive economic energy analysis taking account of environmental benefit.
(14) Availing of training opportunities to personnel at different levels in donor countries and other developing countries to make use of their wide experience in application and commercialisation of renewable energy technologies.
(15) To encourage the private sector to assemble, install, repair and manufacture renewable energy devices via investment encouragement, more flexible licensing procedures.

[VII] CONCLUSIONS

Sudan as an agricultural country has a good rational of energy from agricultural residues, forestry resources, and animal wastes. Sudan has an excellent annual mean solar radiation of 5.44 kW h m^{-2} day^{-1} which could be of strategic important in substituting for oil, electricity, wood and charcoal; in assisting in rural

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development, and in improving the quality of life in rural areas. Sudan is rich in wind; about 50% of Sudan's area is suitable for generating electricity (annual average wind speed more than 5 ms^{-1}), and 75% of Sudan's area is suitable for pumping water (annual average wind speed 3-5 ms^{-1}). Production of bio-fuels such as ethanol from sugar cane, takes advantages of year-round cultivation potential in a tropical country like Sudan. Benefits extend from local to regional to national to global. Local rural economies benefit through new economic opportunities and employment in the agricultural sector. Urban regions benefit through cleaner air and health improvements. The nation benefits through substituting domestic resources for costly imported gasoline. The world benefits from reduced CO_2 emissions.

In a country with a population dense, there are extreme pressures on energy and waste systems, which can stunt the country's economic growth. However, Sudan has recognised the potential to alleviate some of these problems by promoting renewable energy and utilising its vast and diverse climate, landscape, and resources, and by coupling its solutions for waste disposal with its solutions for energy production. Thus, Sudan may stand at the forefront of the global renewable energy community, and presents an example of how non-conventional energy strategies may be implemented.

Air pollution from motor vehicles, electricity generation plants, industry, and other sources, natural and man-made, can harm human health, injure crops and forests, damage building materials, and impair visibility. Public awareness and concern about the problems associated with reduced air quality have increased in recent years. Nevertheless, there still remains considerable uncertainty about both the severity and the valuation of these impacts. The valuation of environmental damages can play an important role in establishing environmental policy and regulatory standards, and can provide guidance in targeting mitigation efforts. In order to achieve environmental objectives at least cost, policy-makers and managers need to balance the relevant social costs and benefits.

The mitigation strategy of the country should be based primarily ongoing governmental programmes, which have originally been launched for other purposes, but may contribute to a relevant reduction of greenhouse gas emissions (energy-saving and a forestation programmes). Therefore, the main fields of emission mitigation will be the energy and the forestry sectors. The main reasons are as follows:

- The overall energy efficiency is far lower than that of the industrialised market economies. The efficiency on both the demands and the supply side has to be increased even in the short run. As far as the supply side is concerned, restructuring of the power plant system is unavoidable, since many plants are old.
- Traditionally, the forestry sector is highly developed and there is enough land even for a larger a forestation programme.

Energy efficiency brings health, productivity, safety, comfort and savings to the homeowner, as well as local and global environmental benefits. The use of renewable energy resources could play an important role in this context, especially with regard to responsible and sustainable development. Implementation of renewable energy technologies offers a chance for economic improvement by creating a market for producing companies, maintenance and repair services.

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Appendix (1) Energy structure in Sudan

Appendix 1.1 Electricity output of present power plants in Sudan (GWh) [28]

Plants	Power (GWh)
Hydro power stations	1095.12
Steam power stations	1037.93
Diesel power stations	359.9
Gas turbine power stations	235.35
Combined power stations	1020.62
Total	3748.9
Peak load for national grid (MW)	534
Total number of consumers (head)	10700000
Total number of employees (head)	7097

Appendix 1.2 Sources of biomass energy available in Sudan 10^6 tons of equivalents (TOE) [29]

Item	Source	10^6 TOE
1.	Natural, and cultivated forests	2.90
2.	Agricultural residues	6.20
3.	Animal wastes	1.05
4.	Water hyacinth	3.16
Total		13.31

Appendix 1.3 Biomass energy consumption in Sudan 10^3 tons of equivalents (TOE) [30]

Item	Sector	10^3 TOE	(%)
1.	Residential	4549	92.0
2.	Industrial	169	3.4
3.	Others*	209	4.6
Total		4927	100.0

*Others are commercial, constructions, and Quranic schools.

Appendix 1.4 Biomass energy potential from animal dung in different states of Sudan [31]

Item	States	Animal dung available (10^3 Tons)	Energy (TOE)
1.	Northern states	102.4	1543
2.	Eastern states	1222.9	18431
3.	Khartoum state	104.3	1572
4.	Central states	4223.7	63658
5.	Darfur states	5062.5	36301
6.	Kordofan states	2596.9	79140
7.	Southern states	4545.2	68505
Total		17857.9	269150

Appendix 1.5 Annual total different biomass supply in regions of Sudan (10^3 kg) [32]

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Regions	Agricultural residues	Animal waste	Bagasse
Northern	288	8548	8644
Eastern	11530	84316	84414
Central	21550	107342	131744
Khartoum	0	5766	5170
Kordofan	2733	234086	236819
Darfur	6901	173376	174067
Southern	1464	290599	197875
Total	44466	904033	838733

Appendix 1.6Annual electricity consumption in Sudan [33]

Sector	Energy	Percent of total (%)
Transportation	3.2	4%
Agricultural	22.4	28%
Industries	6.4	8%
Residential	48.0	60%
Total	80.0	100%

Appendix 1.7 Annual petroleum product consumption in Sudan (10^6 MWh) [34]

Sector	Energy	Percent of total (%)
Transportation	601	60.0%
Industries	138	13.8%
Agricultural	148	14.8%
Residential	55	5.5%
Others*	60	5.9%
Total	1002	100.0%

*Others are commercial and services.

Appendix 1.8 Annual sugarcane bagasse available in Sudan (10^3 tonnes) [34]

Factory	Design capacity	Yearly bagasse
Kenana	300	266
El Genaid	60	53
New Halfa	75	65
Sennar	100	58
Asalaia	100	60
Total	635	502

Appendix 1.9 Per capita consumption for household (10^3 kg) [34]

Fuel	Wood		Charcoal	
Region	Urban	Rural	Urban	Rural
Northern	0.28	0.38	0.83	1.08
Eastern	0.35	0.40	2.21	2.04
Central	0.30	0.58	2.25	2.05
Kordofan	0.41	1.23	5.29	2.30
Darfur	0.41	1.23	5.29	2.30
Khartoum	0.48	0.26	0.93	0.94
Equatorial	0.48	1.40	0.88	0.27
Bahar El Ghazal	0.88	1.36	1.00	0.27
Upper Nile	0.58	0.66	0.76	0.18

Appendix (2) Facts about Sudan [35]

Full country name	Republic of the Sudan.
Total area	One million square miles (2.5 x 10^6 square kilometres). Land 2.376 x 10^6 square kilometres.
Population	35 x 10^6 inhabitants (July 1999 est.).
Capital city	Khartoum (population 5 million).
Language	Arabic (official), English, Nubian, Ta Bedawie, diverse dialects of Nilotic, Nilo – Hamitic, Sudanic languages.
Religions	Sunni Muslim 70% (in north), indigenous beliefs 25%, Christian 5% (mostly in south and Khartoum).
GDP per head	US $ 533.
Annual growth	4% (1997 est.).
Inflation	23% (1998 est.).
Ethnic groups	Black 52%, Arab 39%, Beja 6%, Foreigners 2%, others 1%.
Agricultures	Agriculture is the backbone of economic and social development. 62% of the populations are employed in agriculture. Agriculture contributes 33% of the gross national products (GNP), and 95% of all earnings.
Animal wealthy	35 x 10^6 head of cattle. 35 x 10^6 head of sheep. 35 x 10^6 head of goats. 3 x 10^6 head of camels. 0.6 x 10^6 head of horses and donkeys.
Environment	Fish wealth 0.2 x 10^6 tonnes of food annually. Wildlife, birds and reptiles.
International agreements	Inadequate supplies of potable water, wildlife populations threatened by excessive hunting, soil erosion, and desertification. Party to: Biodiversity, climate change, desertification, endangered species, law of the sea, nuclear test ban, ozone layer protection.

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RESEARCH ARTICLE

PROS AND CONS OF *P. FLORIDA* CULTIVATION FOR MANAGING WASTE OF HANDMADE PAPER AND CARDBOARD INDUSTRIES

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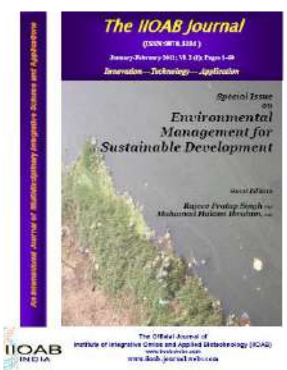
ABSTRACT



The possibility of utilizing handmade paper and cardboard industrial sludges in the production of edible mushrooms involves risk of introducing toxic substances into the human food chain. Therefore, in the present study, genotoxic assessment of P. florida (Pleurotus florida) cultivated on these industrial sludges and their combination with wheat straw was done by Ames test using Salmonella typhimurium TA 98 and TA100. Interestingly, P. florida carpophores, cultivated on wheat straw did not show either frameshift or basepair mutagenicity as revealed by mutagenicity ratio (<2) and mean number of revertants which was found to be 81.3 and 93.4 revertants per plate in the absence of S9 mix. However, this number was found to be increased to 112.1 and 226.3 revertants with S9 mix. P. florida cultivated on waste and its combination showed increase in number of revertants (123.4-170.1 revertants with TA 100 and 79.5-84.1 revertants with TA 98) in the absence of S9 mix over control. Further, increase in number of revertants (229.0-247.3 with TA 100 and 100.3-129.1 with TA 98) was observed on adding S9 mix with both strains S. typhymurium but still mutagenicity ratio was found to be below 2. Hence, these mushrooms were not found to be genotoxic. This mushroom cultivation technique, will not only provide proteinaceous food but also help in reducing industrial wastes. Besides, these can serve as very good source of income for the poor workers working in these industries that can collect the waste from the industries and use it for P. florida cultivation.

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Key words: ***handmade paper industries; cardboard industries waste; sludge; waste management; genotoxicity, mutagenicity***

[I] INTRODUCTION

Waste management services are becoming increasingly important because quantities of wastes continue to raise inspite of waste prevention and cleaner production efforts. Handmade paper and cardboard industries of Sanganer (Jaipur, India) are using colored and white cotton, linen rags and waste paper for making handmade paper and cardboard respectively. These handmade paper and cardboard industries generate colored sludge and brown sludge respectively which accumulates in the vicinity of industries or nearby drains. Non-recycled cellulosic and lignocellulosic pulp fibers are difficult to manage. This industrial waste management is often not only an issue of disposal, but a question of utilizing it for generating wealth from waste. Mushroom cultivation on lignocellulosic and cellulosic industrial wastes, agricultural wastes, agro-industrial sludges are thought to be good techniques to manage waste. It not only removes wastes from the environment but also provide a very good source of protein. This can be very good approach for dealing solid waste especially in developing countries where there is problem of protein deficiency.

Hence, handmade paper and cardboard industrial wastes were used for mushroom cultivation in the previous study [1]. However, it is necessary to analyze the safety aspects of mushroom cultivation before recommending it for consumption especially when industrial waste is used as substrate because mushrooms are well known for their ability to bioaccumulate the toxic substances in their carpophores [2; 3]. In the present study, pros and cons of mushroom cultivation on handmade paper (HMPI) and cardboard industries (CI) waste was analyzed. The present study was thus planned to assess the genotoxic potential of mushroom *P. florida* cultivated on handmade paper and cardboard industrial waste. This will help us not only in generating extra source of income but also providing an attractive way of managing industrial wastes.

[II] MATERIALS AND METHODS

P. florida spawn was purchased from National Research Centre for Mushroom, Chambaghat, Solan. This spawn was used for cultivating mushroom on handmade paper and cardboard industrial waste and their combination with wheat straw (WS) [1].

After harvesting of fruiting bodies (carpophores), aqueous extract of mushroom fruiting bodies was prepared by taking one gram of basidiocarp in homogenizer tube and adding 20ml of sterile distilled water. These samples were homogenized for 10min and raw aqueous extract of mushroom basidiocarps was collected in the tube by filtering the sample using simple filter paper.

Genotoxicity of this aqueous extract were analyzed by *Salmonella*/Microsome reversion assay or Ames assay using *Salmonella typhimurium* TA 98 and TA 100 in the presence and absence of S9 mix [4, 5, 6]. In this study, five dose levels of individual samples (2μl, 5μl, 10 μl, 50μl and 100μl) were tested. The revertant colonies were clearly visible in a uniform background lawn of auxotrophic bacteria revertants appeared on plate were counted for each dose level. Positive controls used for TA 98 and TA 100 were 2- Nitrofluorine (1 μg / plate: 104 revertants) and Sodium azide (1 μg / plate: 594 revertants) respectively. Sterile distilled water was used as negative control.

Analysis of data was done using mutagenicity ratio as described by Mortelmans and Zeiger, 2000 [6]. Mutagenicity ratio is the ratio of average induced revertants on test plates (spontaneous revertants plus induced revertants) to average spontaneous revertants on negative control plates (spontaneous revertants). The following values of spontaneous revertants were obtained for the two strains: Revertant / plate: without metabolic activation TA 98 (28), TA 100 (146); with metabolic activation, slightly higher values were obtained: TA 98 (43), TA 100 (251). Genotoxicity ratio of 2.0 (two fold) or more is regarded as a significant indication of genotoxicity and the compound is considered significantly mutagenic [6]. Experiment was repeated five times and data was pooled and also analyzed statistically by one way ANOVA only at specific dose level i.e. 100 μl.

[III] RESULTS AND DISCUSSION

Results of mutagenicity analysis of *P. florida*, cultivated on industrial sludges and their combination with wheat straw are depicted in Table 1. In this study, aqueous extract of *P. florida* , cultivated on 100% Wheat straw (WS), produced 93 and 81 revertants/ 100 μl of sample with *S. typhimurium* strain TA 100 and TA 98 respectively in the absence of S9 mix. These numbers of revertants were found to increase (TA 100: 226 revertants and TA 98:112 revertants/100 μl of sample) on adding S9 fraction of mouse liver. However, in this case mutagenicity ratios were found to be below 2 indicating that revertants appeared on the plate is mainly spontaneous revertants. Hence, carpophores cultivated on wheat straw were found to be non-mutagenic. In this investigation, these carpophores were used as control for comparing the mutagenicity of *P. florida* cultivated on industrial sludge.

When handmade paper industrial waste alone was used for mushroom cultivation then number of revertants (123 revertants/100μl of sample) was found to increase over control (93 revertants/100μl of sample) with strain TA 100 in the absence of S9 mix. These revertants were found to increase (238 revertants/100μl of sample) on adding hepatic fraction of mouse liver but still aqueous extract cannot be considered as mutagenic due to having mutagenicity ratio below 2. Similarly, aqueous extract of *P. florida* cultivated on 100% HMPI waste did not show frameshift mutagenicity with strain TA 98, in the absence (80 revertants/100μl of sample) and presence (118 revertants/100μl of sample) of S9 mix as revealed by their mutagenicity ratio (<2).

Similarly, on using the waste of cardboard industries alone for mushroom cultivation, aqueous extract of mushroom was showing 170 and 247 revertants/100μl of sample with strain TA 100 in the presence and absence of S9 mix. Again, mutagenicity ratio was found to be below 2 which lead us to conclude that the revertants are spontaneous revertants. However, with strain TA 98, 84 revertants and 129 revertants/ 100μl of sample were found to be appeared on the plate. This case is considered as inconclusive because of having mutagenicity ratio is equal to 2.

In the present investigation, mushroom was also cultivated on combination of wheat straw and handmade paper and cardboard industrial sludge. When 50% WS was mixed with 50%HMPI waste, number of revertants (TA 100: 123 revertants and TA 98: 80 revertants/100μl of sample) was found to increase non-significantly over control but decrease over 100% HMPI waste. Addition of S9 mix was found to increase mutagenicity (TA 100: 238 revertants and TA 98: 118 revertants/100μl of sample) but still mutagenicity ratio was found to be below 2. Hence, mushroom cannot be considered as genotoxic.

On using the waste of 50% CI + 50% WS for mushroom cultivation, aqueous extract of mushroom showed 166 revertants and 79 revertants/ 100μl of sample with strain TA 100 and TA 98 respectively. On adding metabolic activation, these revertants increased to 240 and 122 revertants/ 100μl of sample. Again, mutagenicity ratio was found to be below 2 and differing non-significantly from control and 100% HMPI.

Most of the colored industrial wastes were found to possess mutagenicity. The possibility of utilizing these colored industrial sludges in the production of edible mushrooms implies the risk of bringing mutagenic substances into human food chain. In this study, amazingly, it was found that *P. florida* cultivated on colored handmade paper and cardboard industrial sludge and its combination was found to be non-genotoxic inspite of having colored industrial waste. This may be possibly due to some anti-genotoxic capacity of *P. florida* which has been reported for other mushroom species also including *Pleurotus* [7, 8, 9]. This study shows that *P. florida* cultivated on handmade paper and cardboard industrial waste may be safe for consumption due to having no genotoxicity.

[V] CONCLUSION

Mushroom cultivation is usually rural practice which takes advantage of cheap labour instead of large investment. Labourers and workers working in these industries can collect waste from these industries in spare time and use it for *P. florida* cultivation. Besides reducing the waste, this technology will also provide extra earning to them as Jaipur is center of attraction for tourist who are fond of mushroom and its recepies.

Table: 1. Mutagenicity of aqueous extract of *Pleurotus florida,* cultivated on sludge of handmade paper and cardboard industries alone and its combination with wheat straw

S. No	Substrate used	Dose (in µl)	Raw mushroom extract			
			TA100		TA98	
			S9-ve	S9 +ve	S9-ve	S9 +ve
1	100% WS	2	25.2 ± 2.4 (-)	66.7 ± 5.1(-)	12.1 ± 3.1 (-)	15.1 ± 1.2 (-)
		5	42.1 ± 0.09(-)	91.1 ± 8.6(-)	17.5 ± 4.5 (-)	28 ± 3.2 (-)
		10	65.6 ± 4.8(-)	134.0 ± 12.1(-)	39.8 ± 11.2 (-)	57.0 ± 21.5(-)
		50	88.3 ± 5.4(-)	186.1 ± 13.4 (-)	64.4 ± 15.2 (-)	84.3 ± 18.9 (-)
		100	93.4 ± 3.2(-)	226.3 ± 14.5 (-)	81.3 ± 27.9 (-)	112.1 ± 21.2 (-)
2	100% HMPI waste	2	33.4 ± 1.1(-)	65.3 ± 5.6 (-)	10.2 ± 0.02 (-)	15.3 ± 3.4 (-)
		5	56.3 ± 3.1(-)	103.4 ± 4.5 (-)	24.0 ± 1.09 (-)	32.5 ± 8.9 (-)
		10	87.8 ± 4.5 (-)	146.2 ± 13.1 (-)	46.2 ± 6.5 (-)	55.7 ± 15.6 (-)
		50	98.2 ± 3.8 (-)	182.0 ± 13.2 (-)	68.8 ± 10.8 (-)	75.5 ± 16.8 (-)
		100	123.4 ± 4.2 (-)	238.2 ± 20.8(-)	80.4 ± 14.9 (-)	118.4 ± 23.5 (-)
3	50% WS + 50% HMPI waste	2	28.3 ± 4.5 (-)	58.3 ± 5.6 (-)	9.6 ± 3.4 (-)	11.1 ± 3.6 (-)
		5	45.5 ± 4.4 (-)	87.1 ± 6.8 (-)	20.2 ± 4.5 (-)	23.4 ± 2.5 (-)
		10	77.1 ± 8.6(-)	140.1 ± 13.8(-)	45.2 ± 6.7 (-)	58.1 ± 7.8 (-)
		50	91.3 ± 7.8 (-)	173.4 ± 14.5 (-)	67.1 ± 10.1 (-)	76.8 ± 12.1 (-)
		100	117.3 ± 15.9 (-)	229.0 ± 30.1 (-)	77.4 ± 21.2 (-)	100.3 ± 30.9 (-)
4	100% CI waste	2	42.4 ± 5.6 (-)	50.8 ± 5.6 (-)	16.5 ± 4.5 (-)	20.0 ± 2 (-)
		5	81.3 ± 6.7(-)	97.3 ± 7.6 (-)	27.3 ± 7.9 (-)	43.3 ± 4.1 (-)
		10	110.4 ± 17.8 (-)	116.9 ± 12.1 (-)	40.2 ± 11.3 (-)	66.6 ± 17.8 (-)
		50	146.8 ± 16.6 (-)	195.3 ± 23.4(-)	68.3 ± 13.6 (-)	97.7 ± 14.6 (-)
		100	170.1 ± 23.3(-)	247.3 ± 30.4 (-)	84.1 ± 15.1 (i)	129.1 ± 11.9 (i)
5	50% WS + 50% CI waste	2	35.4 ± 5.4(-)	48.3 ± 4.6(-)	10.1 ± 1.01 (-)	12.5 ± 3.6 (-)
		5	84.2 ± 5.6 (-)	90.1 ± 5.4 (-)	22.3 ± 10.4 (-)	25.4 ± 4.5 (-)
		10	103.4 ± 11.5 (-)	103.4 ± 10.2 (-)	43.3 ± 17.6 (-)	59.5 ± 6.3 (-)
		50	125.8 ± 12.7 (-)	180.5 ± 24.4 (-)	62.5 ± 21.5 (-)	86.3 ± 12.4 (-)
		100	166.1 ± 30.1(-)	240.5 ± 35.1 (-)	79.5 ± 12.8 (-)	122.6 ± 14.8 (-)

Symbols: (-): Mutagenicity ratio below 2 (non-mutagenic); (i): Mutagenicity ratio inconclusive; Numerals are showing mean number of revertants ± Standard deviation; WS-Wheat Straw; HMPI-handmade paper Industries; CI-Cardboard Industries

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REVIEW ARTICLE

EVALUATION OF MOLLUSC AS SENSITIVE INDICATIOR OF HEAVY METAL POLLUTION IN AQUATIC SYSTEM: A REVIEW

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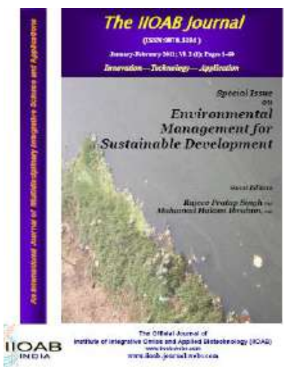
ABSTRACT



A The potential ecological effects of rising levels of heavy metals concentrations in the environment are of great concern due to their highly bioaccumulative nature, persistent behavior and higher toxicity. These chemicals biomagnify in the food chain and impose various toxic effects in aquatic organisms. Molluscs reflect the higher degree of environmental contamination by heavy metals and are the most useful bioindicator tools. Several studies and research work have been cited to establish and evaluate the relationship between metal contents of water column, sediment fractions, suspended matter and mollusc tissue concentrations. The metals body burden in molluscs may reflect the concentrations of metals in surrounding water and sediment, and may thus be an indication of quality of the surrounding environment. The objectives of this work are to gather more information on the use of different species of molluscs as cosmopolitan bioindicators for heavy metal pollution in aquatic ecosystems.

Keywords: ***bioaccumulation; biomonitoring; mollusk; heavy metals***

[I] INTRODUCTION

In the last few decades increasing attention has been paid to the relationship between the conformation of heavy metals and their impact on aquatic organisms. It is widely accepted that anthropogenic activity makes a significant contribution to the total aquatic burden of toxic metals by both point source and non point source contamination can occur. Non point source contamination usually arises from agricultural, industrial, and urban effluents that reach the coast by way of waterways, surface runoff, and precipitation. Both benthic and pelagic species may thus become contaminated by direct uptake and or through biomagnifications. Nevertheless, a permanent control of water quality is indispensable. To reveal the presence of pollutants and to measure their toxic effect biological indicators can be used, which are suitable for prediction of the expectable toxic influence of known or unknown substances.

Metals such as arsenic (As), mercury (Hg), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), manganese (Mn), zinc (Zn) etc. do not degrade in general; therefore, they accumulate throughout the trophic chain. Accumulation in living organisms leads to concentrations several orders of magnitude higher than those of the surrounding water [14]. Despite this the relationship between the concentration of a metal in the aqueous phase and in an organism is far from straight forward as the accumulation ratio depends on many factors; some of them have an environmental origin (temperature, pH, salinity, etc.), whereas others are related to biological factors like age, sex, sexual maturity stage, etc.[40]. Heavy metal toxicity in aquatic organisms, in association with the long residence time within food chains and the potential risk of human exposure, makes it necessary to monitor the levels of these contaminants in marine organisms [28]. In biological indication passive and active monitoring are accepted as a general approach, at different levels of organization. In passive monitoring degradation of the

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ecosystem, elimination of sensitive species and reduction of biodiversity can be revealed as adverse consequences of pollution at the level of populations, while at the level of individuals accumulation of toxic substances in specimen, in organs and tissues indicative of pollution in the environment can be traced. In active monitoring the response of artificial or modified populations, behavioral patterns of specimen, specific function of organs like movement, feeding, respiration, reproduction and the neural regulation as well as cellular and sub cellular events are studied under the effect of toxic substances [57]. To achieve a better estimate of bioavailable metal exposure, it is recommended that the tissues of the organisms be analysed for trace metals [38]. Many benthic organisms accumulate trace metals to the levels reflecting those in the environment. Tissue metal concentrations can reflect contamination, and molluscs in particular may therefore be sensitive biomonitors of anthropogenic metal inputs [32].

[II] HEAVY METALS POLLUTION IN AQUATIC ECOSYSTEM

Aquatic ecosystems are under permanent pressure of anthropogenic pollutants originating from various sources located at the catchment areas, or at distant places. Many of the pollutants are toxic to aquatic organisms causing their lethal or sub lethal deterioration. The toxic effect depends mainly on the type of the pollutant and on its concentration. In most of the cases the concentrations of the pollutants are low, causing only sub-lethal or chronic disease nevertheless, acute massive pollution resulting fish-kill or death of various organisms, may also occur in rivers or lakes. Contamination of aquatic ecosystems (e.g. lakes, rivers, stream, lagoons, oceans etc) with metals has been receiving increased worldwide attention [20, 22, 54].

Heavy metals are a special group of contaminants of water reservoirs. Metals are introduced into the aquatic system as a result of weathering of soil and rocks, volcanic eruptions and from a variety of human activities involving mining, processing and use of metals and/or substances containing metal contaminants [36]. The natural levels of heavy metals in the environment had never been a threat to health but in the recent years increased industrial activities leading to air born emissions, auto exhausts, effluents from industries as well as solid waste dumping have become the sources of large quantities of heavy metals into the environment [39]. Trace metals, when entering into natural water become part of the water-sediment system and their distribution processes are controlled by a dynamic set of physical-chemical interactions and equilibrium. River sediments are basic components of the environment as it provides nutrients for living organisms and serves as sink for deleterious chemical species, reflect the history of the river pollution [55]. Sediments act as both carriers and sinks for contaminants in aquatic environments. Heavy metals are among the most common environmental pollutants and their occurrence in waters and biota indicate the presence of natural or anthropogenic sources. Numerous studies have demonstrated that the concentrations of heavy metals in suspended and bed sediments can be sensitive indicators of contaminants in hydrological systems [20, 33, 35]. The presence of heavy metals in sediments is affected by the particle size, composition of the sediments and other organic substances. The heavy metals can be either adsorbed onto sediments or accumulated in benthic organism, sometimes to toxic levels. Therefore, the mobility, bioavailability and subsequent toxicity of metals have been a major research area [27]. Heavy metals are mainly distributed between the aqueous phase and the suspended sediments during their transport. Riverine suspended load and sediments have important function of buffering heavy metal concentrations particularly by adsorption or precipitation. More than 97% of the mass transport of heavy metals to the oceans is associated with river sediments [34]. Elevated concentrations of trace metals in aquatic bodies as a result of human activities have been recorded since ancient times. However, excessive releases of toxic trace metals into the urban environment and the associated health implications only became apparent in the 1960s when anthropogenic metal contamination of the environment was denoted. Physical mixing of fluvial and marine particulates leads to a continuous decrease in the trace metal content of the suspended matter with increasing salinity. From an environmental and health perspective, this profound geographical development will have a critical influence on our immediate environment and its quality for human health. On a daily basis, numerous human activities including municipal, industrial, commercial and agricultural operations release a variety of toxic and potentially toxic pollutants into the environment [16].

Table: 1. Global emissions of trace metals into the atmosphere, water and soil (in 1000 metric tones yr^{-1}) [44].

(1) Metals	(2) Air	(3) Water	(4) Soil
(5) Arsenic	(6) 18.8	(7) 41	(8) 82
(9) Cadmium	(10) 7.6	(11) 9.4	(12) 22
(13) Chromium	(14) 30	(15) 142	(16) 896
(17) Copper	(18) 35	(19) 112	(20) 954
(21) Mercury	(22) 3.6	(23) 4.6	(24) 8.3
(25) Indium	(26) 0.02	(27) -	(28) -
(29) Manganese	(30) 38	(31) 262	(32) 1670
(33) Molybdenum	(34) 3.3	(35) 11	(36) 88
(37) Nickel	(38) 56	(39) 113	(40) 325
(41) Lead	(42) 332	(43) 138	(44) 796
(45) Antimony	(46) 3.5	(47) 18	(48) 26
(49) Selenium	(50) 3.8	(51) 41	(52) 41
(53) Tin	(54) 6.4	(55) -	(56) -
(57) Thallium	(58) 5.1	(59) -	(60) -
(61) Vanadium	(62) 86	(63) 12	(64) 132
(65) Zinc	(66) 132	(67) 226	(68) 1372

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During recent years, the pollution of reverine system by heavy metals has attracted a lot of attention of the scientific community. Unlike organic pollutants, natural processes of decomposition do not remove heavy metals. On the contrary, they may be enriched by organisms and can be converted to organic complexes, which may be even more toxic. The metal solubility is principally controlled by pH, concentration and type of ligands and chelating agents, oxidation-state of the mineral components and the redox environment of the system. Since each form may have different bioavailability and toxicity, the environmentalists are rightly concerned about the exact forms of metal present in the aquatic environment. The toxicity and fate of the water borne metal is dependent on its chemical form and therefore quantification of the different forms of metal is more meaningful than the estimation of its total metal concentrations. Critical assessment of the endpoints of determination for potentially and actually available and accessible metal fractions in the environmental matrices of water, soil, and sediment become the basis for a need-specific monitoring strategy [45]. Several studies has been conducted to reveal the heavy metals concentrations in river water and sediments throughout the world. Heavy metal concentrations in sediment are many times greater than the same metals in the water column. Sediments can act as a scavenger agent for heavy metal and an adsorptive sink in aquatic environment. It is therefore considered to be an appropriate indicator of heavy metal pollution [33]. River water and sediment were assessed for metal and nutrient concentrations from the cities in the Pearl River Delta, South China and observed that sediments were seriously contaminated with Cd, Pb, and Zn in accordance with the classification by Hong Kong Environmental Protection Department [16]. The magnitude and ecological relevance of metal pollution of the middle Po river deriving from the River Lambro tributary was investigated for the partitioning patterns of target heavy metals (Cd, Cu, Ni, Pb, Zn) as well as by investigations of Total metal concentrations in the surface sediments revealed significant pollution inputs on the whole river stretch investigated, with a distinct peak at the inlet of the River Lambro [22]. Different statistical techniques are also being applied for the detailed evaluation of spatial and temporal variations in water quality and different heavy metals. River Gomti monitored at eight different sites in relatively low, moderate and high pollution regions, regularly over a period of 5 years (1994–1998) for 24 parameters [54], Karoon river for Ni, Cr, Cu and results revealed that the minimal and maximal concentrations of these metals in winter were 69.3–110.7, 1.7–118.3, and 5.5–70.3 g/l, for Ni, Cr, and Cu, respectively. The minimal and maximal concentrations of these metals in spring were 41.0–60.7, 0.7–19.8, and 0.5–28.7 g/l, for Ni, Cr, and Cu, respectively [20]. The measurement of total metal may not be able to provide information about the exact dimension of pollution in riverine systems, thus the determination of different fractions assumes great importance. Several researchers studied the distribution of several trace metals in surface water and sediments by leaching, extraction and ion exchange speciation processes as well as fractionation of metal ions on bed sediments of river [35, 55].

[III] NEED OF BIOMONITORING OF AQUATIC ENVIRONMENT

Chemical analysis of the environment matrix such as water, sediment is the most direct approach to reveal the heavy metal pollution status in the environment, while it cannot afford the powerful evidence on the integrated influence and possible toxicity of such pollution on the organisms and ecosystem. Biomonitoring is a scientific technique for assessing environment including human exposures to natural and synthetic chemicals, based on sampling and analysis of an individual organism's tissues and fluids. The results of these measurements provide information about the amounts of natural and manmade chemicals that have entered and remained in the organisms and the corresponding effects induced. Due to consistency between the selected organisms and the corresponding living space, biomonitoring can directly offer the data on the potential effects and actual integrated toxicities of pollutants, reflecting the corresponding deleterious degree in the environment [65].

An important approach to assessment of risk from environmental and occupational exposures is biomonitoring which provides an estimate of the total dose absorbed and gives indirect access to determination of target site concentrations. To reveal the presence of pollutants and to measure their toxic effect biological indicators can be used. Active and passive monitoring are two general approaches to assess the pollutants and their toxic effects at different levels from species to community level of any ecosystem. In passive monitoring degradation of the ecosystem, elimination of sensitive species and reduction of biodiversity can be revealed as adverse consequences of pollution at the level of populations, while at the level of individual's accumulation of toxic substances in specimen, in organs and tissues indicative of pollution in the environment can be traced. In active monitoring the response of artificial or modified populations, behavioral patterns of specimen, specific function of organs like movement, feeding, respiration, reproduction and the neural regulation, as well as cellular and subcellular events are studied under the effect of toxic substances.

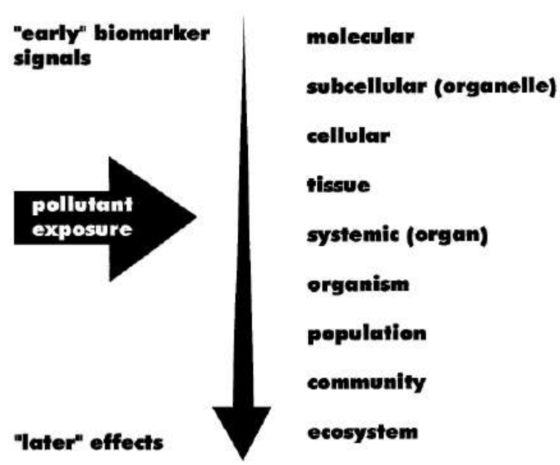

Fig: 1. Schematic representation of the sequential order of responses to pollutant stress within a biological system [51].

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It is stated that an organism is a product of its environment and hence has an indicator value. A bioindicator is defined as a plant or an animal which reveals the presence of a substance in its vicinity by showing some typical symptoms which can be distinguished from the effects of other natural or anthropogenic stresses [39]. A good bioindicator is one which shows the earliest responses to the pollutants enabling to indicate the presence and predict the consequences of undesirable anthropogenic effects [52]. In biomonitoring surveys, the toxic elements arsenic, cadmium, chromium, cobalt, lead and nickel etc are used as examples to illustrate the disturbing factors in the interpretation of biomonitoring results [17]. The accumulation of trace elements in aquatic consumers is of interest to environmental scientists concerned with the fate and effect of contaminants, as well as to ecologists interested in food web dynamics and trace metal biogeochemical cycles to assess the toxic impact or distribution of contaminants [43]. It is necessary to understand how elements move through aquatic food webs. Understanding the means by which aquatic organisms accumulate trace metals from their environment is complicated by the existence of both soluble and dietary sources. For many aquatic invertebrates, trophic transfer accounts for a major portion of total trace element accumulation [1]. In the field, the ecotoxicological approach is very difficult for evaluation of the impact of heavy metals in an aquatic environment, due to the complexity of interrelationships between organisms and the ecosystem. However, field studies can enable assessment of the long-term effect on organisms of heavy metals. The underlying regulator of metal concentrations accumulated by animals in tissue is the balance between accumulation and elimination (both of which vary according to the organism's accumulation strategy and diluting body growth) [18, 56].

Trace metal exposure may induce specific metal-binding ligands. Other ligands such as sulfide are important for Ag biokinetic changes in bivalves. Metals also interact strongly in their accumulation by aquatic animals. Generally, dissolved Hg uptake is reduced following exposure to other metals such as Ag, Cd, Cu, and Zn in mollusks and invertebrates. The tissue body burden and the detoxificatory fate of metals in animals seem to be more important in affecting metal accumulation than the nature of the exposure routes (aqueous vs. dietary) or of the exposure regimes. Trace metal accumulation may also be variable in different natural populations of bivalves as a result of different physicochemical environments and histories of exposure [34].

Assessing bioaccumulation is also a component of international efforts to identify and control chemicals of environmental concern. It is now generally accepted that substances, which are persistent, bioaccumulative, and toxic and are subject to long range transport are of particular concern. There is a need to establish reliable procedures for estimating bioaccumulation potential from knowledge of molecular structure or from readily measurable properties of the substance. There is a further inventive to adopt a tiered assessment system in order that those substances which are not bioaccumulative can be rejected from the assessment process at an early stage with minimal expense and effort. Even toxic effects on ecosystems start with these chemical reactions in individuals. In past few years monitoring programs, conducted to evaluate water quality, usually include chemical and common biological parameters, the use of biochemical markers for surveys is less frequent, but recently more efforts have been given to propose these biomarkers of exposure and effect, in toxicity testing aiming an application in pollution monitoring [29].

[IV] FACTORS AFFECTING BIOACCUMULATION OF HEAVY METALS IN MOLLUSCS

Different environmental factors such as water current, water flow, renewal of water, pH, hardness, salinity etc greatly affects the distribution of heavy metals in the molluscs. The greater metabolic rate of small organisms may partially account for the higher concentration of the essential elements Cu and Zn. [64]. Body size and weight also play an important role in bioaccumulation of metals. Sex plays a significant role in metals accumulation in molluscs. Significantly lower concentrations of cadmium and zinc concentrations were found in males as compared to females in Perna perna species and high concentration of cadmium was reported in Pecten maximus [10, 50]. Metal concentration may differ according to the species. Uptake of metals and subsequent bioavailability are highly dependent on geochemical and biological factors. Among biological factors, there are major differences in bioaccumulation between bivalve species. Within a single species accumulation can be a function of age, size, sex, genotype, phenotype, feeding activity and reproductive state [8].

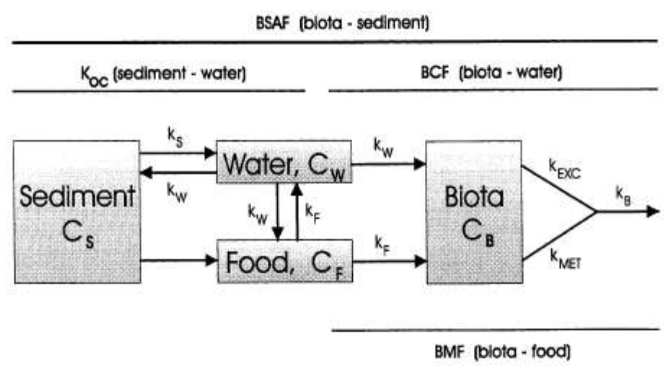

Fig: 3. Bioaccumulation model for aquatic organisms. KOC: sorption coefficient; BCF: bioconcentration factor; BSAF: biota-sediment accumulation factor; BMF: biomagnification factor. C refers to a concentration and k to a rate constant. The subscripts S, W, F, B, EXC and MET refer to sediment, water, food, biota, excretion and metabolism, respectively. The digestible sediment fraction is considered to be part of the food [62].

Geochemical factors that influence bioaccumulation are organic carbon, water hardness, temperature, pH, dissolved oxygen, sediment grain size, and hydrologic features of the system [21]. Salinity influences availability of contaminants whereas

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temperature changes can alter bivalve spawning, which will affect the soft tissue mass and lipid composition of the mollusc [6]. Uptake and accumulation in deposit feeders would be expected to correlate to metal concentrations in sediments, whereas accumulation in filter feeders would most likely reflect metal concentrations in water [42]. Various workers noticed that in oysters, blood amoebocytes have been reported to present membrane-limited/membrane-bound vesicles, which can trap copper and zinc [26, 59]. In bivalves, metallothioneins may be trapped in whole soft tissues. Induction of metallothioneins binding cadmium in various soft tissues, gills, labial pulps, digestive gland as well as in remaining tissues, have been studied by several researchers [5,6]. High environmental phosphate concentration facilitates the uptake of cadmium by organisms [50]. Antagonism between zinc and cadmium has been reported on many organisms. However, the concentration of metal in the molluscs depends not only on the level of the element in the environment but also on other factors such as size, age, speed of growth, sex and reproductive conditions of the molluscs, season, salinity, chemical species and interaction with other pollutants.

[V] SIGNIFICANCE OF MOLLUSCS OVER OTHER AQUATIC INVERTIBRATES

The use of aquatic organisms as bioindicators for trace metal pollution is very common these days. Molluscs are among the organisms most used for this purposes. It is widely observed that various species of molluscs are the effective sentinel organisms and can achieve high concentrations of metals and metalloids relative to concentrations gradient of these substances in the surrounding environment [47]. Biomagnification in molluscs and most of the other aquatic animals involves the uptake of chemical from the water and sediments. Bioaccumulation is the process, which causes an increased chemical concentration in aquatic organisms compared to that in water, due to uptake by all exposure routes including dietary absorption, transport across respiratory surfaces and dermal absorption. Bioaccumulation can thus be viewed as a combination of bioconcentration and food uptake. Biomagnification can be regarded as a special case of bioaccumulation in which the chemical concentration in the organism exceeds that in the organism's diet due to dietary absorption. The extent of bioaccumulation thus can play key role in determining water and sediment quality criteria. The assessment of the levels of heavy metals pollution in aquatic molluscs which are used as bioaccumulation indicators, has become an important task in preventing risks to public health. It is to be pointed out that for a living species to be used as bioaccumulator some essential characteristics are necessary (i) It must be typical of the ecosystem studied (eg non-migratory), ubiquitous and abundant, (ii) Its size, biotype and behavior must be such as to make sapling easy. (iii) It must bioaconcentrate xenobiotics substances to a level sufficient to perform a direct analysis without pre-concentrations. (iv) It must be able to stand high concentrations of different toxic substances so as to survive the pollutant studied. (v) It lives in a sessile style, thus definitely representing the local pollution. (vi) Its life long enough for the comparisons between various ages. (vii) It occupies the important position in food chain. (viii) Dose effect relationship can be observed in it [46, 65].

Among aquatic organisms, gastropods and bivalves molluscs have been recognized as a useful tool for monitoring of the environment they live in because of their ability to accumulate chemical elements and/or compounds in their tissues proportionally to their bioavailability and thus can be used as indicators of aquatic metallic pollution. They are filter feeders, herbivores or carnivores and have the potential to bioconcentrate contaminants, which would normally be present in the water or within sediments at concentrations too low for detection by routine monitoring techniques. They are also ideal species for environmental monitoring, because their sedentary nature does not require consideration of complex migratory factors in the interpretation of the bioaccumulation data. They are sedentary organisms filtrating large amounts of water allowing them to accumulate the substances from the environment. They also satisfy the other conditions to be bioindicators hence very appropriate for monitoring because of their abundance and wide geo- graphical distribution, relative longer life span, suitable dimensions, size, weight, easy identification and collection, abundance in an ecosystem and accumulate the elements to a degree suitable to measure for hazard and risk assessment [15]. Molluscs are also sturdy enough to survive in laboratory and field studies and tolerant to environmental alterations, and various contaminants [65].

[VI] BIOMONITORING OF HEAVY METAL POLLUTION USING GASTROPODS AND BIVALVES

Benthic molluscs play an important role as bioindicators for trace metal pollution and appear more and more often in global monitoring programs [47]. Among aquatic organisms suitable for biological monitoring molluscs occupy a prominent place and they are often used both for passive and active biomonitoring and in hazard and risk assessment [52]. In recent years, researchers have focused their attention on the identification of other possible bioindicators for trace metal pollution, such as the gastropod molluscs. Several biomonitoring studies for the heavy metals pollution in aquatic ecosystem, have been carried out in past two decades using different mollusk species like, gastropod mollusc Bembicium nanum [25], Donax trunculus and Chamelea gallina [61], bivalve: Pyganodon grandis [9], Crassostrea angulata, Scrobicularia plana, Palameon longirostris, Uca tangeri, Melicertus kerathurus [7], Crassostrea virginica [3], Radix ovata and Viviparus spp. [31], Rapana venosa and Neverita didyma [37]. This is because it is necessary to identify a wider range of bioindicators and thus expand current understanding of different bioaccumulation strategies for trace metals.

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Both essential and non-essential trace elements are known to be highly accumulated by invertebrates, in particular by a variety of molluscs species. Aquatic molluscs seem to reflect ambient metal contamination and are therefore widely used as bioindicator organisms. Undoubtedly, aquatic molluscs are amongst the most thoroughly investigated bioindicator organisms. The translocation of sentinel species, mainly mussels from a reference site to the study areas has been demonstrated as a useful strategy for the assessment of water quality in coastal and estuarine environments, either through bioaccumulation or biomarkers analysis [41, 19, 31]. Body size, condition index and tidal height also affects the concentrations of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in Mytilus edulis and findings suggested that the body weight was inversely related to metal concentrations and for Cd, Mn, Pb and Zn the regression was affected by tidal height. Except for As, Fe and Mn metal concentrations were inversely related to physiological status though no differences between essential and non-essential metals were recorded. Thus it is recommended that stringent measures during sampling for biomonitoring or metal concentrations at each location must be normalized to a common body size, condition index and tidal height [40]. As even closely related species may exhibit different accumulation strategies for trace elements, there is a need to identify widespread cosmopolitan biomonitors to allow intra-specific comparison of accumulated metals concentrations over large geographical areas [47]. Considering that bioaccumulation of heavy metals is highly site dependent, it was of general interest to test the suitability of molluscs as metal bioaccumulators in the moderately polluted waters. Different feeding habits in mollusc may influence metal bioaccumulation for example the prosobranch grazer Viviparus species is both a deposit-feeder and facultative suspension-feeder. In this species, the bypobranchial gland beneath the gill coats the filtered particles with mucus, food particles are then transported towards the mouth and ingested [31].

Various metal accumulating bivalve and gastropod species show a high presence and abundance in marine and freshwater reverine ecosystems therefore they are suitable for different monitoring projects for example Cd, Cr, Zn in Perumytilus purpuratus, Semelle solida and Tagellus dombeii [30]. Mussels can accumulate and integrate concentrations of several metals in seawater for relatively long intervals. They also assimilate trace metals from their food and from the ingestion of inorganic particulate material [46]. Moreover, bivalves such as Mytilus galloprovincialis [24]; Perna perna [23] and oysters eg Crassostrea angulata [24]; Crassostrea virginica [56] are widely employed in laboratory as well as field studies where the uptake, loss or the biological effects of heavy metals such as arsenic (As), Lead (Pb), Cromium (Cr), Manganese (Mn), Copper (Cu), Zinc (Zn) on the east coast of the middle Adriatic Sea [33], Black sea [49]; Monodonta turbinata and Patella caerulea in Mediterranean area [12], Mytilus edulis, Crassostrea talienwhanensis and Ruditapes philippinarum along the Chinese Bohai Sea [37] and results showed that Crassostrea talienwhanensis possessed a much greater ability for bioaccumulation of Cu and Zn than other species Rapana venosa manifested the high bioaccumulation capacity of Cd. Among the five species, the Ruditapes philippinarum possessed the highest content of Ni. Furthermore, Cd, Cu and Zn contents in some gastropods and oysters samples exceeded the maximum permissible levels established by WHO. Another species Mytilus trossulus and the Barnacle balanus are also being used for such type of metals biomonitoring [47]. The digestive gland of bivalves is a target organ for the accumulation of metals, furthermore, the lysosomes of the digestive cells are generally considered as target organelles. While the gills have also been shown to accumulate various heavy metals either in the field or in the laboratory. A preliminary investigation conducted to assess the pollution in the northern part of Vietnam with focus on trace elements including heavy metals in a freshwater bivalve Pletholophus swinhoei used for human consumption. Significant site-specific differences were reported for As, Ba, Be, Br, Cr, Fe, Mn, Ni, P and Sr [63]. Freshwater mussel Dreissena polymorpha also used to assess trace metal pollution in the lower river Po, Italy and results showed that the Cd and Pb concentrations were increased from 1.23 to 3.22 and 3.40 to 5.93 pg/g dry weight respectively at one site and the same trend was observed at the second site, indicating that these metals accumulated. Cu and Zn concentrations in mussel tissues did not change relative to time zero concentrations. The highest Cr and Ni levels were found after 15 days at both stations (10 and 27 pg/g dry weight respectively). Low and relatively constant Hg concentrations (<0.10 pg/g dry weight were found in transplanted and native molluscs (D. polymorpha and Unio elongatulus). Concentration factors calculated for all trace elements assayed, ranged from 103 (Pb) to 104 (Zn) [13].

Environmental pollutants such as metals pose serious risks to many aquatic organisms. Accordingly, a great deal of previous research that has characterized physiological mechanisms of toxicity in animals exposed to contaminants. Relationships between ambient geochemistry, watershed land-use and trace metal (Cu, Zn, and Pb) concentrations in three types of invertebrate aquatic molluscs, odonates, and composite were established [48]. The results suggest that despite the high variation in ambient metal concentrations within each land-use category, macro invertebrates in ponds were accumulated higher levels of Cu and Zn but the levels of Cu, Zn, and Pb in invertebrates from all ponds were less than dietary concentrations considered toxic to fish. Relationship of Cd, Pb, Zn, Cu, Ni, Co, Cr, Mn and Fe in the soft tissue of Turbo coronatus, Acanthopleura haddoni, Ostrea cucullata and Pitar sp., as well as in associated surface sediments (bulk and bioavailable metal concentrations) from the Gulf of Aden, Yemen were showed the significant spatial differences in metal concentrations in the molluscs and associated sediments. A slope of the linear regression was noted significantly higher than unity for Fe (9.91) and Cd (3.45) in A. haddoni and for Ni (4.15) in T. coronatus, suggesting that the bioavailability of these metals is disproportionally increased with a degree of enrichment of the sediments in Fe, Cd and Ni, respectively. A slope constant approximating to unity (1.14) for Cu in A. haddoni relative to its concentration in sediment extract implies that bioavailability of this metal proportionally increased with growing concentrations of its labile forms in the associated sediment [58].

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Metallothioneins are cysteine rich, low molecular weight, heat stable proteins that bind to metals such as Cd, Cu and Zn [53]. Metallothioneins in aquatic invertebrates play an important role in the homeostasis of essential metals like Cu and Zn and detoxification of excess amount of essential and non essential metals such as Cd, therefore extensively used as as biomarkers [2, 47]. Moreover, MT levels are also known to be related to the fitness status and health of organisms [11]. The literature on metallothioneins (MT) and metallothionein-like proteins (MTLP) in aquatic invertebrates is large and increasing. Metallothionein like proteins appear to play an important role in mediating metal uptake and hence accumulation, therefore metallothionein has been assayed in a range of aquatic animal tissues as an indicator of metal exposure [4, 2]. MTs can be induced by the essential metals Cu and Zn and the non-essential metals Cd, Ag and Hg in both vertebrates and invertebrates, but their induction is variable [60]. Against this background of variability MTs do appear to play roles both in the routine metabolic handling of essential Cu and Zn, but also in the detoxification of excess amounts intra-cellularly of these metals and of non-essential Cd, Ag and Hg. Different isoforms of MT play different physiological roles, and the dependence on MT in detoxification processes varies environmentally and between zoological groups.

[VII] CONCLUSION

Various species of gastropods and bivalves molluscs have been recognized as a useful tool for monitoring of heavy metals pollution. These organisms accumulate comparatively higher concentrations of metals because of their sedentary nature, both from water and sediment. The extent of bioaccumulation thus can play key role in determining water and sediment quality criteria. Molluscs occupy the important position in food chain and are ubiquitous and abundant, live in a sessile style thus represents the pollution level of habitat. Several studies have been carried out on its different life stages including embryonic stages. They also satisfy the other conditions to be bioindicators like their abundance and wide geo- graphical distribution, relative longer life span, suitable dimensions, size, weight, easy identification and collection. There are suitable to measure for hazard and risk assessment. Molluscs are also sturdy enough to survive in laboratory and field studies and tolerant to environmental alterations, and various contaminants.

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