*International Research Journal of Environmental Sciences*_____ ISSN 2319–1414
Vol. **6(7),** 1-8, July **(2017)** *Int. Res. J. Environmental Sci.*

Bio-medical waste (BMW) generation and their effects on adjacent environment

Sudhir K. Upadhyay[1*], Garima Singh[1], Abhishek K. Srivastava[2] and Devendra P. Singh[3]
[1]Department of Environmental Science, VBS Purvanchal University, Jaunpur-222003 India
[2]Department of Biotechnology, MHPG College, Jaunpur-222003 India
[3]Department of Environmental Science, BBAU, Lucknow India
sku.env.lko@gmail.com

Available online at: www.isca.in, www.isca.me
Received 11th February 2017, revised 26th June 2017, accepted 10th July 2017

Abstract

The present study was based on BMW from Health Care Units (HCUs) at Jaunpur city-India, during 2013 to 2015 year. Out of 48 HCUs, maximum BMW (37.2 Kg day^{-1}) was generated by orthopedic-HCUs followed by surgical, gynecology and cardio; while total BMWs generation was about 98 Kg day^{-1} and mostly BMW were dumped in low line area. Results of physio-chemical, elemental and bacterial analysis of BMW-leachate were significantly influenced by seasonal variation. Concentration of metals were observed as Ca>Cu>N≈P>K>Zn>Cr>Pb>Hg in almost all types of BMW leachate. M. luteus *was found with maximum frequency (42%) in Gyneic-BMW followed by E. coli, Pseudomonas, S. aureus, B. subtilis and K. pneumonia. Fifty percent of fish mortality was found at lethal dose (LD_{50}) of 30%, 25%, 22% and 18% of BMW-leachate (w/v) after 24, 48, 72 and 96h respectively. Contamination of heavy metals to aquifer by BMW - leachate were observed in adjacent area through Hand Pump water (HPW) analysis and results revealed that more concentration of Ca, Cu, Cr, Zn, and Pb were found as compared with control. No significant (P=0.5%) difference were observed among 100-250m and 250-500m HPW samples for Ca, Cu and Pb, except Cr. BMW dumping sites and adjacent HPW showed more concentration of heavy metals than of WHO recommendation. In discriminate disposal of BMW could have disastrous for human being along with adjacent environment.*

Keywords: BMWs generation, Toxic metals, Bacteria, Season variation, Aquifer contamination.

Introduction

Improper management of waste generated through health care units (HCUs), commonly known as Bio-medical Waste. BMW mostly contains infectious agents, hazardous chemicals/metals, pharmaceuticals, radioactive materials, living tissues etc., which generated through treatment of patients as well as biomedical research activities[1,2]. Sometimes infectious component mixed with the non-infectious component in BMW, then the entire mass increase their infectious potentialities[3]. Management of BMW from hospitals, houses, streets, shops, offices and industries was the responsibility of municipal or governmental authorities, but now it has become mandatory for hospitals, clinics, other medical institutions and veterinary institutions to dispose of BMW as per the Law[4], however well documented instruction for management of BMW disposals are reported in guidelines of WHO[5]. Several infectious diseases are spread by open dumping of BMW like hepatitis, HIV, gastro enteric infections, respiratory infections, blood stream infections, skin infections, radioactive toxicity etc, along with BMW also contaminate air, soil and water[1]. Open dumping of BMW/Landfills have been identified as one of the major threats to groundwater resources, Salami et al. earlier reported that dumping sites of wastes have more responsible for ground water pollution through leachates formation[6]. The impact of landfill on the surface and groundwater was earlier reported in last decads[6-8] and most of them assess the contamination of underground water by leachates percolation and their estimation through mathematical modeling[6,9]. Generation of leachates are mainly due to rain fall on the top of the waste landfill sites or sometimes surface runoff[10], after few days inorganic and organic matters of BMW are discomposes or degraded and form several gaseous and other products like methane, ammonium, carbon dioxide, organic acids, aldehydes, alcohol, free available form of metals *ie* calcium, magnesium, sodium, potassium, iron, sulphates, chlorides, cadmium, mercury, chromium, copper, lead, nickel and zinc etc[11]. Thus ways, contamination of groundwater poses a substantial risk to local resource user as well as natural environment[12,13]. Leachates of landfill are contain dissolved organic matters, inorganic compounds, such as etc. Excess nutrients such as nitrates and phosphates from BMW/landfills can cause a phenomenon called eutrophication (when surface of the water body develops algal blooms) in stagnant water, can alter the pH, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) and finally damage entire aquatic ecosystem. The management systems of BMW in India are actually not upto international standard[5]. District Jaunpur is a historical city of Uttar Pradesh – India, situated at 25.44^0N, 82.41^0E. Males and female population are 53% and 47% respectively with high population density.

There are no proper disposable methods of BMW by HCUs in district Jaunpur, therefore huge amount of BMW waste are being dump on low line area. Keeping these views; the objective of the present study was to estimate the load of BMW at Jaunpur city generated by HCUs and their effects on adjacent environment.

Materials and methods

Study sites of HCUs and calculation of BMW generation: A descriptive cross- sectional study based on self-administered questionnaire among HCUs in Jaunpur city on the provisions of BMW (management and handling) Rules, 1998 led to revelation of some significant observation with respect to the collection, storage, transportation, treatment and disposal of BMW, during the year of 2013 to 2015. A random descriptive cross- sectional study was carried out each year and study was based on about 38 doctors, 145 nurses and 350 health professionals employed at the hospitals. The amount of waste generated in kg day^{-1} in each hospital was determined as and recorded from each day over a three month period (a season to year). Standard statistical deviations $\sigma_n - 1$ of parentheses data form samples were calculated as[14].

$\sigma_n - 1 = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$. The arithmetic means x was determined as $(\sum x)/n$, where n is the number of days. Projections for the average quantity of various types of waste generated computed from the data.

Sample collection, analysis of physico-chemical, elemental and bacterial study: Two types of samples were taken in present study, (i) BMW-Leachate and (ii) Hand pump water (**Figure-1**). Leachate samples were collected from the base of BMW heaps where the leachate was drained out by earth's gravity. Out of fifty-two hand pumps, 15 and 13 were around 100-250meter and 250-500 meter apart from chosen BMW dumping site (site was about 7 year old), water samples from hand pumps were taken weekly. Eighteen hand pumps were apart from about 1.5 to 2 Km from dumped sites of BMW taken as a Control. Both types of sampling were carried out in summer, rainy and winter season (year 2013 to 2015) form district Jaunpur -India. Various physico-chemical parameters like viz Colour, pH, ECe (electrical conductivity), BD (bulk density), Nitrate (NO_3^-), Total Phosphorus (TP), Total potassium (K), Calcium (Ca), Copper (Cu), Chromium (Cu), Zinc (Zn), Mercury (Hg) and Lead (Pb) were determined by using Atomic Absorption Spectrophotometer (AAS) through digestion of sample by tri-acid mixture consisting of HNO_3, H_2SO_4 and $HClO_4$ in the ratio 9:2:1[15,16]. Ten gram leachate sample was added to 90 ml of sterile distilled water and shaken for 30 min on a mechanical rotary shaker. Ten fold dilutions were made and plated on to three different media, Nutrient Agar, King's B and McKonky agar medium, and incubated at 38 ± 2^0C[17]. Single bacterial colonies were isolated after 24h. Bacterial strains were morphologically identified using gram staining reaction and other biochemical tests which include; catalase, methyl red-voges proskauer (MR-VP), nitrate reduction test, starch hydrolysis, gelatin liquefaction test, coagulase, indole, motility, oxidase, urease, triple sugar iron agar (TSI) and sugar fermentation as described by Ogbulie et al.[18] and Cheesbrough et al.[19]. Microorganisms were enumerated as described by Oyeleke et al. [20].

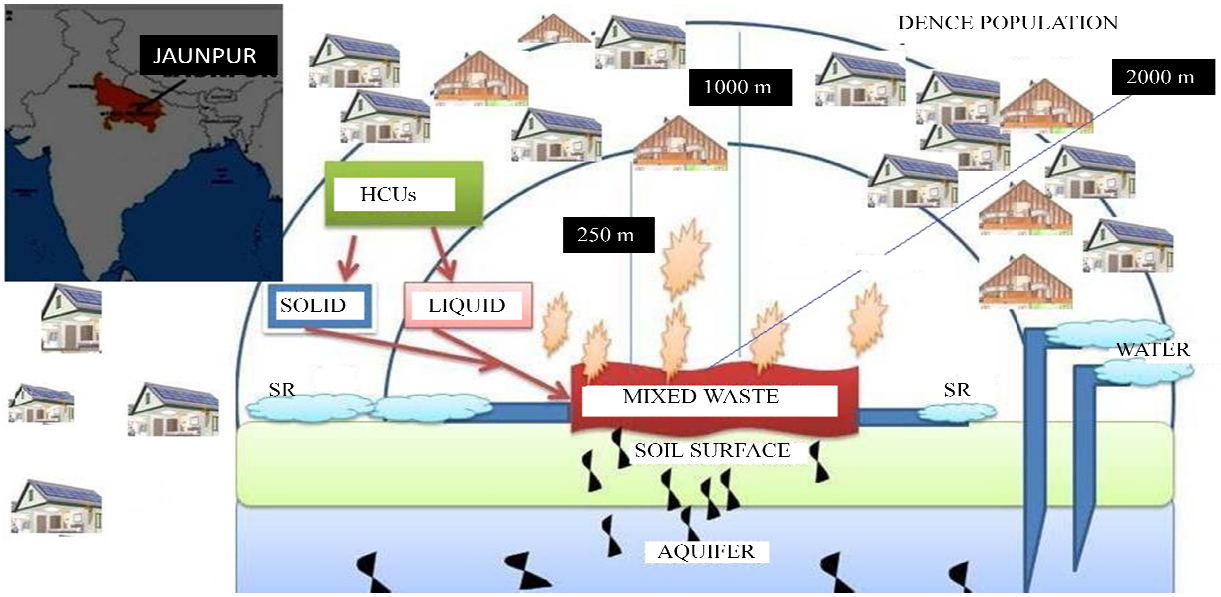

Figure-1: Schematic diagram of effects of open dumping sites of BMW generated by HCUS in Jaunpur District-India, and hand pump water (aquifer water) near 100M to 2km from BMW dumping sites. BMW=Bio medical wastes, HCUs=Health care units, SR=surface run-off and =Leachate/chemicals/elements.

Bioassay test for leachate of BMW: Different concentration [10 to 100% (w/v)] of Leachate samples were prepared with sterile distilled water in a tank (Volume 10L), without concentration of leachate in tank taken as a control. Twenty similar sizes of *Cyprinus carpio* fishes were kept in each tank for 96hour and observed their mortality rate; as per standards of Environmental Protection Act rule for waste water 90% of fishes were survive after 96 hour. The *Cyprinus carpio* fish having commercial importance is a known bioindicator used in the toxicological tests[21]. This test is recommended as a general Standard test by Environmental Protection Act-1986.

Results and discussion

Study sites of HCUs and calculation of BMW generation: Total 48 health care units (HCUs) were used in the present study among the Jaunpur city, India. HCUs were varied with respective practices like Gynecology (GN), Orthopedic (OR), Dental (DN), General surgery (SG), Cardio (CD), Pediatric (PD) and Pathology (PT). Maximum numbers of HCUs were PT and OPD (*ie* 19) followed by SR, CD and PD (*ie* 5 each), then GN (*ie* 4 each), least numbers of HCUs were for OR and DN (*ie* 3 each) data not shown. Result revealed from average three seasons of two year study, the maximum amount (37.2 Kg day^{-1}) of BMW was generated from OR-HCUs followed by CR, SG and GN. Least amount of BMW (0.8 $Kgday^{-1}$) was generated by DN-HCUs. While about 98 Kg day^{-1} of BMW was generated by all HCUs trough out the year. Thus this situation is really an alarming and inducing load of BMW in native area, it could be manage through skilled medical staff/personnel engaged in the process of segregation, handling and transport of BMW[4]. The waste was segregated separately, according to its characteristics, at the point of generation, mainly from the patient care areas, separation of BMW are based on their specific nature[4]. In the present study, maximum HCUs of district Jaunpur-India were not strictly follow the BMW handling rules[4], therefore maximum amount of BMW were deposited on open dumping sites. In this connection; present situation of study area were highlighted earlier by first author in news paper of the country. More of the HCUs deposited their BMWs at their near area, because there has lack of sincerity along with no any proper facilities to dispose for BMW like incinerator etc.

Physio-chemical, elemental and bacterial analysis of BMW: All the BMW samples were collected from open dumping sites of respective HCUs and they were shown variation in the results of physio-chemical, elemental and bacterial observation over the seasons. More basic pH and high electrical conductivity (ranged from 12 to 3.0 dSm^{-1}) was observed in summer session followed by winter and rainy (Table-1a,b,c) These high values could be due to the effect of the concentration of salts as a consequence of degradation of organic matter[22] and these wastes could be responsible for develop a barren land *ie* ECe $\geq$4dSm^{-1} [23]. In the present study, varied physicochemical properties were observed in samples of BMW sites with the range of BD [2.1 to 1.7 (gm cc^{-3})] and OC (6.2 to 2.6 %) over the sessions; due to organic carbon contents, moister, rainfall and microbial interactions alters the of samples characteristics of BMW[2,24] reported medical waste have varieties of metals and other hazardous products, In the present study, result revealed over two year observation the concentration of Ca was found to be maximum and rest of the metals were having following trends Cu>N≈P>K>Zn>Cr>Pb>Hg (Table-1a,b,c) among the all types of BMWs. Concentration of heavy metals in a landfill is generally higher at earlier stages because of higher metal solubility as a result of low pH caused by production of organic acids[25]. Copper and Zinc was observed in more concentration during summer season followed by rainy and winter about all types of BMW except Cr, Zn, Hg, and Pb (Table-1a). The concentration of Cr was relatively more in rainy season followed by summer and winter, and it was more in waste of GN-HCUs followed by the waste of SG, DN and OR- HCUs (Table-1b). Maximum Hg concentration (19 ppm) was observed in the waste of DN followed by GN-HCUs during rainy season (Table-1b). Similar finding was earlier reported for Hg metal in dental amalgam[26]. Lead was observed in the waste of GN,OR and SG HCUs, and it was more in summer followed by rainy and winter season (Table-1a,b,c). Heavy metals were found in most of the BMWs samples sites around over all three seasons and they were more than the permissible range of WHO recommendation[27]. In the present study, maximum bacterial population was observed in rainy followed by summer and winter season among the waste of different HCUs. Maximum (Cfu: 83 X $10^7 gm^{-1}$ BMW) bacterial population were observed on MacConkey agar plate after 24h followed by NA (Cfu: 66 X 10^7 gm^{-1} BMW) and King'S B medium (Cfu: 42 X 10^7 gm^{-1} BMW) during rainy session (Table-1a,b,c). *Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Pseudomonas, and E. coli* isolates were observed in the samples of all types of BMW (Biochemical Characterization data not shown). All the bacterial isolates were observed maximum in their frequency during rainy season among all the BMWs. Isolates of *K. pneumonia* and *Pseudomonas* were not detected in OP waste, while maximum isolates of *E.coli* were found among all types of BMWs. *Micrococcus luteus* were found with maximum frequency (42%) in GN-BMWs followed by *E. coli, Pseudomonas, Staphylococcus aureus, Bacillus subtilis* and *K. pneumonia* (Table-2). BMW from PD had maximum frequency of K. *pneumonia* bacterial isolates followed by *Micrococcus luteus*. Maximum frequency of *E. coli* isolates were found in OR, DN, SG and CR-wastes followed by *Staphylococcus aureus* (Table-2). Microbes grow with a very fast rate and contaminate adjacent environment through surface run-off and presence of pathogenic microbes in open dumping of BMW sites are cause of several disease[28,29]. In spite of that microbes have capabilities to induce the elements/chemicals transformation in BMW and form new hazardous products/chemicals, and such hazardous products could caused several disorder in human beings as well as adjacent environment through surface runoff/leachets[30]. Hazardous products/chemicals were leached and contaminated to aquifers water[12,13]. In the present study, OC, N, P and K and

heavy metals were observed in more concentration in BMW and these elements leads to eutrophication in water body[24], as well as chances of aquifer contaminations could be increased through the formation of leachets respectively[30].

Table-1: Physico-chemical, elemental and biological status of different types of BMW samples taken during (2013-2015 YEAR).

Types of BMWs	Table (1a) Summer sessions																	
	Physico chemical analysis					Elements in BMW-Leachate (ppm)									Bacterial population (Cfu:10^6/gm of BMW)		
	pH	Color	BD	OC	ECe	Ca	N	P	K	Cu	Cr	Zn	Hg	Pb	NA	KBA	. MCA
GN	7.8	DB	1.8	6.2	12	135	155	86	67	54	1.8	19	3.0	6.1	49	32	63
AR	7.5	LG	2.1	4.0	9.0	121	22	215	32	22	ND	12	ND	8	12	6.0	26
DN	7.1	LG	1.7	5.1	11	117	18	124	33	15	0.9	ND	15	ND	2.0	1.0	17
SG	7.5	B	1.9	2.7	8.0	101	216	104	33	43	1.9	11	ND	12	38	22	42
CR	7.6	LG	1.7	4.2	3.0	22	26	22	64	22	ND	13	2.0	10	9.0	3.0	11
PD	7.8	B	1.8	4.3	11	112	22	43	33	32	ND	12	ND	0.8	7.0	2.0	8.0
PT	7.3	LG	1.7	2.6	5.0	97	103	102	12	41	ND	11	4.0	4.2	21	14	25
Table (1b) Rainy session																	
GN	7.2	LG	1.9	7.2	21	131	76	36	22	43	8.2	13	3.6	1.4	66	42	83
OR	2.0	LG	2.8	5.2	10	120	32	27	36	32	1.7	2.1	ND	2.2	45	12	22
DN	7.1	LG	1.8	5.6	9.1	67	22	111	41	9	1.2	0.8	19	2.0	62	22	52
SG	7.7	B	2.3	3.2	11	11	201	22	42	62	2.5	22	ND	6.0	22	18	27
CR	7.2	LG	1.9	4.2	8.2	19	33	13	22	14	ND	ND	ND	ND	29	21	28
PD	7.9	B	1.9	4.7	2.7	82	27	76	29	52	ND	8.0	ND	ND	61	16	17
PT	7.1	B	1.8	3.1	7.1	22	98	82	41	24	ND	4.2	2.0	ND	28	8	22
Table (1c) Winter Session																	
GN	7.7	LG	2.2	6.1	20	111	23	24	22	35	1.3	2.4	2.0	1.3	46	11	56
AR	7.0	LG	2.1	5.5	11	120	32	25	31	30	1.2	ND	ND	1.5	56	14	28
DN	7.8	LG	1.5	5.7	2.8	62	18	101	23	31	ND	1.7	14	1.2	37	13	12
SG	7.3	B	2.1	3.8	13	09	217	34	28	11	1.5	2.1	ND	1.9	29	10	13
CR	7.5	LG	1.9	4.1	18	11	42	25	31	13	ND	6.0	ND	ND	78	13	12
PD	7.6	B	1.9	4.2	12	34	26	45	22	17	1.0	1.5	ND	ND	22	8	21
PT	7.9	B	1.4	3.2	9.4	14	37	42	15	10	1.6	2.2	ND	ND	61	10	10

Data are average mean of ∑60 samples (5 samples/week upto 3 months for each session) of BMW of respective Health Care Units (HCUs). GN=Gyanic, OR=Orthopediatric, DN= Dental, SG=General surgery, CD=Cardio, PD= Pediatric and PT= Pathology, DB= Dark brown, LG=Light gray, G=gray, B=Black, BD=Bulk density (g/cc^3), OC=Organic Carbon in %, ECe=Electrical conductivity, NA=Nutrient agar, KB= King'S B, MCA=MacConkey agar and Cfu=Colony forming Unit, and ND=Not detected.

Table-2: Frequency (percentage (%) of appearance) of bacterial isolates among BMWS of respective health care units (HCUS).

HCUs	*K. pneumonia*			*Bacillus subtilis,*			*Staphylococcus aureus*			*Micrococcus luteus*			*Pseudomonas*			*E. coli*		
	S	R	W	S	R	W	S	R	W	S	R	W	S	R	W	S	R	W
GN	5	11	18	14	8	6	15	11	12	34	42	22	10	12	20	22	16	22
AR	ND	3	9	25	31	7	27	19	24	10	8	11	ND	11	21	39	26	28
DN	ND	12	ND	ND	9	ND	25	17	21	19	22	25	22	12	22	34	28	32
SG	7	12	6	9	8	14	32	20	32	20	12	18	10	18	4	22	30	26
CR	11	19	5	7	7	22	22	24	13	18	20	10	12	10	10	30	30	40
PD	24	27	32	ND	ND	ND	25	13	12	11	25	16	22	15	18	18	20	22
PT	9	22	18	8	2	17	22	24	15	21	16	10	12	15	12	28	15	28

Data are average mean of ∑60 samples (5 samples/week upto 3 months for each session) of BMW of respective Health Care Units (HCUs). GN=Gynecology, OR=Orthopedic, DN=Dental, SG=General surgery, CD=Cardio, PD=Pediatric, PT=Pathology, S=Summer seasons, R=Rainy seasons, W=Winter sessions and ND=Not detected.

Evaluation of hazardous effects of BMW in adjacent aquifer: Keeping this view, in the present study we examined the heavy metals concentration among the different HPW (hand pump water), which were 100, 250, 500m, 1000m and 2000m apart from BMW dumping site, results revealed that more Ca, Cu, Cr, Zn, and Pb were found in 100-250m followed by 250-500m compared with control. No significant (P=0.5%) difference were observed in 100-250m and 250-500m water samples for Ca, Cu and Pb, while significant difference was observed in the case of Cr (Figure-2). As par WHO guidelines; Cu, Cr, Zn and Pb were more the permissible limits except Ca near area of BMW dumping sites. This showed an alarming situation for human being who live near area of BMW dumping sites. Cu, Cr, Zn are an integral part of many important enzymes involved in a number of vital biological processes[31-34] while their high concentration and long term exposure could cause several disorder like oxidative stress etc.[32-34]. The reduction of Cr(VI) to Cr(III) results in the formation of reactive intermediates that together with oxidative stress oxidative tissue damage and a cascade of cellular events including modulation of apoptosis regulatory gene, contribute to the cyto-toxicity, genotoxicity and carcinogenicity of Cr(VI)-containing compounds[32,35]. On the other hand, chromium is an essential nutrient required to promote the action of insulin in body tissues so that the body can use sugars, proteins and fats. Chromium is of significant importance in altering the immune response by immune stimulatory or immunosuppressive processes as shown by its effects on T and B lymphocytes, macrophages, cytokine production and the immune response that may induce hypersensitivity reactions[32]. Lead is not reported for their vital role in biology and exposure to lead produces various deleterious effects on the hematopoietic, renal, and reproductive and central nervous system, mainly through increased oxidative stress[36]. Plum et al.[34] reported high-dose zinc supplementation and long term exposure interferes with the uptake of copper. Hence, many of its toxic effects are in fact due to copper deficiency. While systemic homeostasis and efficient regulatory mechanisms on the cellular level generally prevent the uptake of cytotoxic doses of exogenous zinc, endogenous zinc plays a significant role in cytotoxic events in single cells. Here, zinc influences apoptosis by acting on several molecular regulators of programmed cell death, including cascade and proteins from the Bcl and Bax families[34]. One organ where zinc is prominently involved in cell death is the brain, and cytotoxicity in consequence of ischemia or trauma involves the accumulation of free zinc. Rather than being toxic metal ions, zinc is an essential trace element[34].

Toxicity test of BMW- leachate: In the present study, toxic effects of BMW-*leachate* were observed in fish population upto 96h in ambient condition (Figure-3) and result revealed about fifty percent of mortality was found at lethal dose (LD_{50}) of 30%, 25%, 22% and 18% of BMW-*leachate* (w/v) after 24, 48,72 and 96h. BMW-*leachate* showed adverse effect on *survivability* of fish population. There have been several reported cases of fish mortality due to the discharge of industrial effluents from several industries into the receiving water bodies[37,38]. The pollutants build up in the food chain are responsible for the adverse effects and finally death of aquatic organisms[30].

Conclusion

From this study, we conclude that - i. In discriminate disposal of BMW from HCUs are damage the soil land, could be induce in spreading of pathogenic microbes, ii. open dumping sites of BMW could be contaminated aquifer through leachates and

adjacent environment through surface run-off, iii. the concentration of heavy metals in sites of BMW samples and adjacent hand pumps water were more than the WHO recommendation. Therefore, In discriminate disposal of BMW could have disastrous for human being along with adjacent environment.

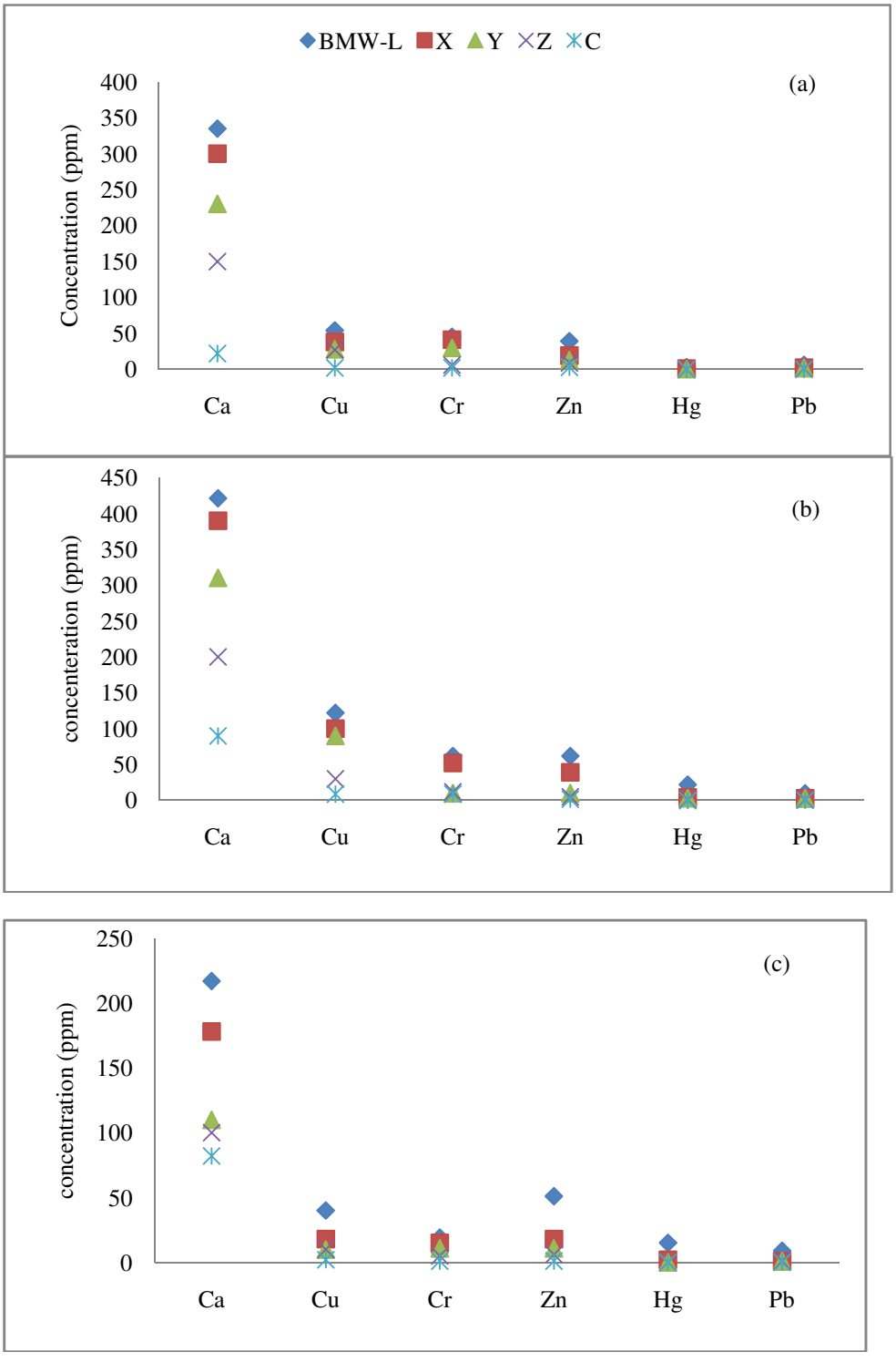

Figure-2: Heavy metals concentration in hand pump water (a) Summer (b) rainy and (c) winter seasons; BMW-L (BMW leachate), X=100-250m, Y=250-500m, Z=500-1000m and C (control) =1000-2000m.

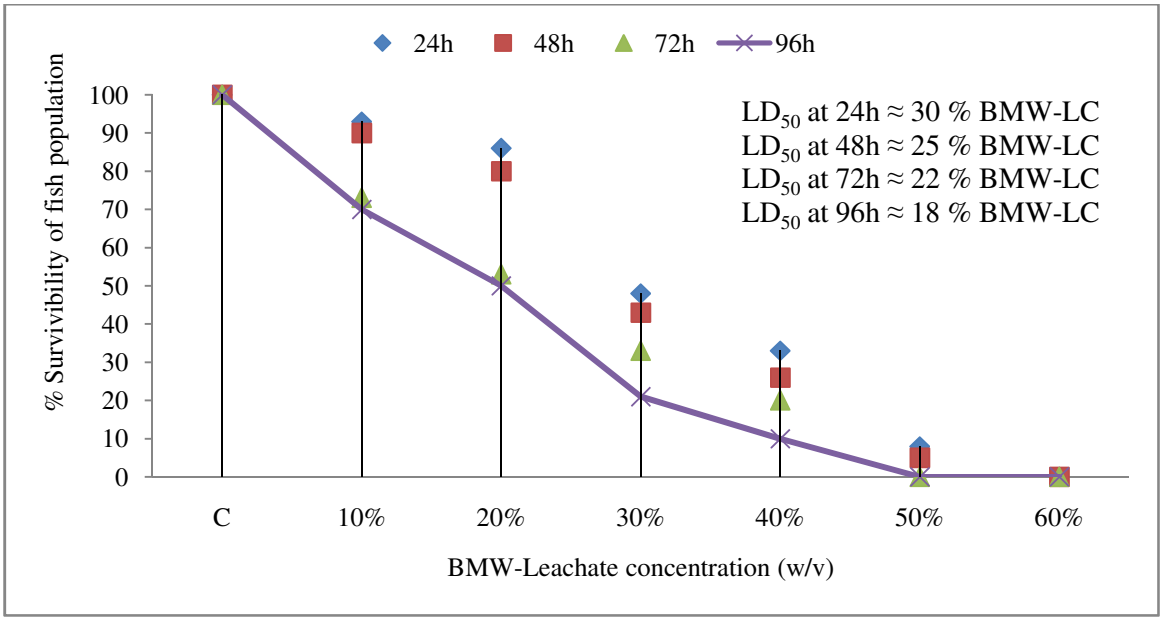

Figure-3: Percent survivability of fish population under different concentrations of BMW- Leachate [BMW-LC (w/v)] and LD_{50}= Lethal dose of BMW-LC to kill 50% population of fish.

References

1. Baveja G., Muralidhar S. and Aggarwal P. (2000). Hospital Waste Management – an overview. *Hospital Today,* 5-9, 485-486.

2. Rutala W.A., Weber D.J. (2014). Disinfection, sterilization and control of hospital waste. Mandell, Douglas and Bennett. Principles and practice of infectious diseases (6th ed.), *Elsevier Churchill Livingstone Publication*, 3331-3347.

3. Nugget Info (2003). Hospital Waste Management and Bio-degradable Waste. *Government of India*, Press Information Bureau, http://pib.nic.in/infonug/infaug.99/i3008991.html – downloaded on 18.05.2003.

4. Notification: Bio-medical Waste (Management and Handling) Rules, 1998. Ministry of Environment and Forests, GOI (E), part 3(ii), New Delhi. 27.07.1998.

5. Biomedical Wastes, http://www.swachdelhi.comNew.htm; 2004.

6. Salami L., Olafadehan O.A., Babagana G. and Susu A.A. (2013). Prediction of concentration profiles of contaminants in groundwater polluted by leachates from a landfill site. *International Journal of Research and Reviews in Applied Sciences*, 15(3), 365-378.

7. DeRosa E., Rubel D., Tudino M., Viale A. and Lombardo R.J. (1996). The leachate composition of an old waste dump connected to groundwater: Influence of the reclamation works. *Environmental Monitoring Assessment*, (40), 239-252.

8. Saarela J. (2003). Pilot investigation of surface parts of three closed landfills and factors affecting them. *Environmental Monitoring Assessment*, 84(1-2), 183-192.

9. Moo-Yound H., Johnson A., Carson D., Lew C., Liu S. and Hancock K. (2004). Characterization of infiltration rates from landfills: Supporting groundwater modeling effort. *Environmental Monitoring Assessment*, 96(1-3), 283-311.

10. Jhamnani B. and Singh S.K. (2009). Groundwater contamination due to Bhaswa landfill site in New Delhi. *International Journal of Civil and Environmental Engineering*, 1(3), 121-125.

11. Renou S., Givaudan J.G., Poulain S., Dirassouyan F. and Moulin P. (2008). Landfill leachate treatment review and opportunity. *Journal of Hazardous Materials*, 150(3), 468-493.

12. Mor S., Ravindra K., Dahiya R.P. and Chandra A. (2006). Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site. *Environmental Monitoring and Assessment*, 118, 435-456.

13. Ogundiran O.O. and Afolabi T.A. (2008). Assessment of the physicochemical parameters and heavy metal toxicity of leachates from municipal solid waste open dumpsite. *Int. J. Environ. Sci. Tech*, 5(2), 243-250.

14. Gupta S.P., Statistical Methods, 24th ed. Revised & Enlarged S. Chand & Sons, (1998), New Delhi. 110 002.

15. APHA (2005). Standard methods for the examination of water and waste water. 20th Edn. APHA, AWWA, WPCF, Washington, USA.

16. Mani A.K., Santhi R. and Sellamuthu K.M. (2007). A handbook of laboratory analysis. 1st edition, A.E. Publications, Sundaram, P.N. Pudur, Coimbatore, 10, 156-167.

17. Upadhyay S.K., Singh D.P. and Saikia R. (2009). Genetic Diversity of Plant Growth Promoting Rhizobacteria Isolated from Rhizospheric Soil of Wheat under Saline Condition. *Current Microbiology*, 59(5), 489-496.

18. Ogbulie J.N., Uwazuoke J.C. and Ogieho S.I. (1998). Introductory Microbiology Practical. Springfield Publishers Nigeria, 70-120.

19. Cheesbrough M. (2003). Water quality analysis. District Laboratory practice in Tropical countries (2) Cambridge University Press. United Kingdom, 146-157.

20. Oyeleke S.B. and Istifanus N. (2009). The microbiological effects of hospital wastes on the environment. *African Journal of Biotechnology*, 8(22), 6253-6257.

21. ISO 7346/1: Water quality- Determination of acute lethal toxicity of substances to a freshwater fish (Brachydanio rerio Hamilton-Buchanan (Teleostei, Cypinidae) – Part 1: Static method 1984.

22. Francioso O., Ciavatta C., Sanche-Cortes S., Tugnoli V., Sitti L. and Gessa C. (2000). Spectroscopic characterization of soil organic matter in long-term amendments trials. *Soil Sci.*, 165(6), 495-504.

23. Upadhyay S.K. and Singh D.P. (2015). Effect of salt-tolerant plant growth-promoting rhizobacteria on wheat plants and soil health in a saline environment. *Plant Biology*, 17, 288-293.

24. Correll D.L. (1998). The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review. *Journal of Environmental Quality*, 27, 261-266.

25. Kulikowska D. and Klimiuk E. (2008). The effect of landfill age on municipal leachate composition. *Bioresource Technology*, 99(13), 5981-5985.

26. Drummond J.L., Cailasb M.D. and Croke K. (2003). Mercury generation potential from dental waste amalgam. *J Dentist,* 31, 493-501.

27. WHO (2004). Guidelines for Drinking Water Quality. 3rd Edn., 1, Recommendation, Geneva, 515.

28. Anonymous: Categorization of biological agents according to hazard and containment. 4th ed. Second supplement (1995). Available from: URL: http://www.hse.gov.uk.hthdir/noframes/agent/pdf.

29. Anonymous (2000). Guidelines for protecting the safety and health of health workers. NIOSH / Health Care Workers guidelines. National Institute for Occupational safety and Health, Washington DC: US Government office, Chap 6.

30. Ogundiran M.A., Fawole O.O., Adewoye S.O. and Ayandiran T.A. (2010). Toxicological impact of detergent effluent on juvenile of African catfish (*Clarias gariepinus*). *Agric. Biol. J. N. Am*, 1(3), 330-342.

31. Hingston J.A., Collins C.D., Murphy R.J., and Lester J.N. (2001). Leaching of chromate copper, arsenate wood preservatives, a review. *Environmental Pollution*, 111(1), 53-66.

32. Shrivastava R., Upreti R.K., Seth P.K. and Chaturvedi U.C. (2002). Effects of chromium on the immune system. *Pathogens and Disease*, 34(1), 1-7.

33. Gaetke L.M. and Chow C.K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology,* 189(1-2), 147-163.

34. Plum L.M. and Haase R.H. (2010). The Essential Toxin: Impact of Zinc on Human Health. *Int. J. Environ. Res. Public Health*, 7(4), 1342-1365.

35. Stohs S.J., Bagchi D., Hassoun E. and Bagchi M. (2001). Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J. Environ. Pathol. Toxicol. Oncol*, 20(2), 77-88.

36. Flora G., Gupta D. and Tiwari A. (2012). Toxicity of lead: A review with recent updates. *Interdiscip Toxicol*, 5(2), 47-58.

37. Adewoye S.O., Fawole O.O., Omotosho J.S. and Owolabi O.D. (2005). Toxicity of cassava a waste water effluents to African catfish (*Clarias gariepinus*). *Ethiop. J. Sci,* 28(2), 189-194.

38. Roopadevi H. and Somashekar R.K. (2012). Assessment of the toxicity of waste water from a textile industry to Cyprinus carpio. *J. Environ. Biol*, 13, 167-171.