Toxicity evaluation of MSW leachate using *Oncorhynchus mykiss* and *Gambusia affinis* acute toxicity tests in Kermanshah, western Iran

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Abstract

Various physical, chemical and biological characteristics of Kermanshah municipal solid-waste leachate including COD, BOD, EC, pH, T, TSS, TDS, NO_3^--N and PO_4^{-3}-P were determined. Laboratory analysis revealed that samples collected in November-January when precipitation is on its peak are highly polluted with very high TSS (9398.8 mg L^{-1}), BOD (2630 mg L^{-1}) and COD (6240 mg L^{-1}), NO_3^--N (4100 mg L^{-1}), PO_4^{-3}-P (62.4 mg L^{-1}). The toxicity of leachate produced at the Kermanshah municipal solid waste dump was determined using the 96-h acute toxicity test with *Oncorhynchus mykiss* and *Gambusia Affinis*. Result of toxicity assay indicates that the leachates were highly toxic to *Oncorhynchus mykiss* (LC50=6.07% v/v) and *Gambusia affinis* (LC50=12.41% v/v).

Keywords: leachate, toxicity test, Oncorhynchus mykiss, Gambusia affini.

1 Introduction

Leachate created by municipal solid waste is a highly complex mixture of organic and inorganic compounds [1]. Using acute and chronic toxicity tests with various animals and plants several studies have shown that leachates produced by municipal solid wastes are highly toxic [2]. Although unpredictability of interactions between components of the leachate poses difficulties in the

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interpretation of the results of such toxicity tests, gross toxicity testing of mixed chemicals is still a valuable tool for screening the possible risk to the environment. Identifying contaminants responsible for toxicities is difficult because of the restricted number of chemicals detected by routine analysis, the complexity of the leachate mixtures and the uncertainty surrounding their bioavailability. The impact of the leachate produced in landfills or in garbage dumps on plants and animals is considered to be high and is believed to be caused by several factors, such as high load of organic matter, heavy metals toxicity, high content of nitrogen and other transported contaminants [3]. Municipal solid waste leachates created from open garbage dumps can be a major source of contamination to groundwater and surface waters. In an open dump leachate can easily be exposed to various segment of the ambient environment through uncontrolled overflow, rainfall, run off, subsidence and infiltration [4].

There are many problems associated with municipal and hazardous solid waste in Iran. Although by law regulatory arrangements control the disposal of various solid wastes in many developing countries including Iran, municipal solid waste are mainly deposited in open dumps. Municipal solid waste generally consists of wastes generated from residential and commercial areas, industries and urban streets [5]. Such indiscriminate complex of unwanted materials is not properly sorted at the source, but collected together in waste bins. Environmental condition resulted from climate, topography, hydrology and hydrogeology of the dumping site together with the nature of the waste may further characterize the dump and its leachate. Preliminary attempts to recycle and recover materials from garbage dumps in the metropolitan areas in Iran have failed to establish a reliable strategic policy for an integrated solid waste management. However, there are increasing attentions to the problems associated with solid waste management in metropolitan areas in Iran. The annual waste generation increases in proportion to the rises in population and urbanization in small cities. Generally lower standard of living in these cities produce more organic waste, such as kitchen wastes, and fewer recyclable items, such as paper, metals, and plastics. Such a combination of waste produce considerable amount of leachate which become a new environmental problem [1]. Meanwhile, rapid development and changing lifestyles in large and medium size cities in Iran also influence the composition of the solid waste from mainly organic to mainly plastics, paper, and packaging materials that are complex in nature. In the absence of almost any sanitary landfill in Iran there are open dumps for all human settlement which by virtue of its population may have a chance to have a regulatory body for collection and transportation of the municipal solid wastes.

The large volume of municipal solid waste (MSW) of growing population in Kermanshah together with many hazardous materials which produce by various industrial sectors inside the city or in the industrial parks are dumped in Sarab Ghanbar. This mixture poses a serious environmental threat to surface and groundwater reserves. The present study was conducted to provide a preliminary evaluation of the environmental hazards the Kermanshah MSW dump to the ambient environment.

2 Materials and methods

2.1 Sample collection and analysis

Kermanshah, a medium sized city in western Iran with a population of over 600000, has a garbage dump located in Sarab Ghanbar over the top of a valley (Fig 1) approximately 7 Kilometers in western part of the city. Kermanshah municipal garbage dump is an open dump which receives various domestic and hazardous waste for over two decades. Presence of constant observable volatile organic compounds (VOCs) including methane (with or without flame) indicates that the landfill is in its methanogenesis stage and receives fresh organic compounds regularly. Gradual accumulation of organic debris over 20 years has a produced a great mound of garbage with a stream of constant flow of leachate at the bottom of the valley with varying amount of discharge corresponding to the seasonal precipitation (Fig 2).

Leachate samples were collected monthly during November, December 2007 and January 2008. Composite samples for chemical analysis were prepared and replicate measurements were made on each sample as described for the individual methods. The samples were stored in air-tight 1-L polyethylene and glass bottles. They were transported to the laboratory and frozen until needed. Individual containers were defrosted when required, and COD, BOD5 and other analyses were carried out. Freezing kept the samples COD and BOD5 constant. Therefore, the leachate used in this study had an invariable 6249 mg/L COD and

Figure 1: Kermanshah garbage dump in western part of the city in Sarab Ghanbar.

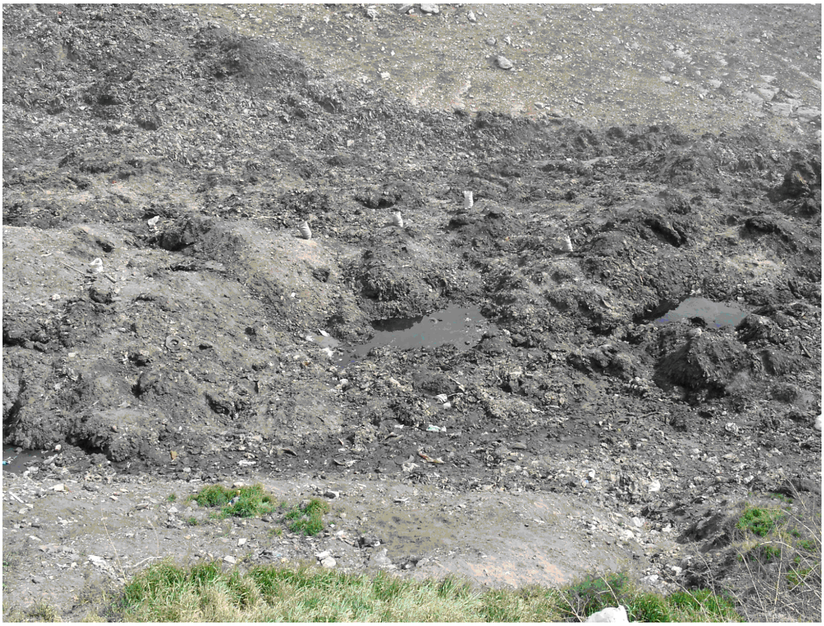

Figure 2: Leachte created at Kermanshah garbage dump.

2630 mg/L BOD5. Unused, defrosted leachate was immediately discarded. Leachate samples were analyzed for pH and electrical conductivity (Jenway 4330 pH and Electrical Conductivity). Chemical oxygen demand (COD) and biological oxygen demand (BOD) were determined using standard methods for the examination of water and wastewater, 1998 [13]. Samples were digested with concentrated nitric acid (Method 3030E, APHA, 1995). Total phosphate determined using the mixed acid digestion, molybdenum blue method. Nitrate nitrogen determined using nitrate electrode connected to 701A ionalyzer.

The organism chosen for this evaluation was Rainbow trout (*Oncorhynchus mykiss*) and Gambusia fish (*Gambusia Affinis*). Fingerling of rainbow trout (about 10 grams), *O. mykiss*, were bought and transformed from a fish farming unit in an aerated plastic bag. Adult Gambusia fish (*Gambusia affinis*) were caught by hand net in a karst spring in Chehr area in eastern Kermanshah and brought to laboratory. Fish bioassay was performed for these species of fish using five concentrations (0.9, 1.8, 3.6, 7.2 and 14.4 v/v) of Kermanshah MSW leachate (COD=6250 mg/l) in separate 10-L static aquariums. Five separate jars were prepared to conduct the bioassay tests. The jars with plastic led used to provide single aeration system connecting air tubes via hole made on the plastic lids. Fish were maintained in two big and separate tanks prior to the onset of experiment for two weeks. Fish were feed every other day during the adaptation period and the feeding stopped two days before the experiments. Following preparation of diluted leachate 10 rainbow trout were added to each of the five treatments in triplicates and the number of death and environmental condition of such as temperature, dissolved oxygen and pH were recorded at 2, 4, 6, 8, 24, 48, 72 and 96 h during the bioassay. Similar toxicity tests were arranged with *Gambusia affinis* with 20 fish in every jar. Room temperature (25±1°C) and light/dark cycle (16 hr light/8 hr dark) were kept constant. LC50 values and their ±95% confidence limits were calculated using Probit Analysis software downloaded from the US-EPA site.

3 Results

3.1 Physico-chemical analysis

Values of physico-chemical parameters of Kermanshah MSW leachate is shown in are being discharged. The average pH value of 6.9 in an arid environment in which pH for surface water is normally over 8 indicates the role of decomposition of organic materials in characterizing leachate reaction. Although the leachates obtained from a single outlet at the Kermanshah MSW dump during winter time there was considerable inter-sample variability. Excess of organic and mineral in the leachate is extraordinary high and are indicated in Table 1. Both the organic content and the nitrogen content (NO_3^--N=4100 mg/L) were typically high. Soluble phosphate (PO_4^{-3}-P=64.2 mg/L) in comparison to soluble nitrate is low and may be due to the excess of organic content of the leachate absorbing soluble phosphate.

Table 1: Physico-chemical characteristics of the Kermanshah MSW dump leachate collected in winter with toxicity values.

Parameter	Unit	Kermanshah MSW leachate
pH		6.99
EC	µS cm−1	336
TSS	mg/L	9398.9
TDS	mg/L	1908
NO_3^--N	mg/L	4100
PO_4^{-3}-P	mg/L	64.2
BOD	mg/L	2630
COD	mg/L	6240

3.2 Bioassay

The results for 96 hour acute toxicity tests for *Oncorhynchus mykiss* and *Gambusia affinis* are shown respectively in Table 2 and 3. Lethal concentration (LC50) for Kermanshah MSW leachate and their lower and upper 95% confidence limits for both species are shown in Table 4. The average LC50 for both *Oncorhynchus mykiss* (6.07%v/v) and *Gambusia affinis* (12.4% v/v) showed that the Kermanshah MSW leachate is highly toxic. The large difference in the responses of these two fish can be attributed to the differences in their sensitivity to environmental condition as *Oncorhynchus mykiss* is known to be a stenotype species well adapted to cold and well oxygenated freshwater, while *Gambusia affinis* is an euroytpic species originated from tropic freshwater adapted to a wide range of variation in water temperature, salinity and oxygen content.

Table 2: Results of bioassay of the leachate from Kermanshah MSW to rainbow trout, *Oncorhynchus mykiss*.

Concentration v/v%	No of fish	No of dead fish during the experiment (h)					
		6	8	24	48	72	96
14.4	10	2	4	6	7	8	9
7.2	10	0	0	1	2	3	4
3.6	10	0	0	1	1	2	3
1.8	10	0	0	0	0	1	2
0.9	10	0	0	0	0	0	0

Table 3: Results of bioassay of the leachate from Kermanshah MSW to *Gambosia affinis*.

Concentration v/v%	No of fish	No of dead fish during the experiment (h)					
		6	8	24	48	72	96
14.4	20	0	6	8	10	10	11
7.2	20	0	0	1	3	4	6
3.6	20	0	0	0	1	1	3
1.8	20	0	0	0	0	0	1
0.9	20	0	0	0	0	0	0

4 Discussion

Chemical characterization of organic and inorganic compounds in MSW leachate is not analytically straightforward. As the result quantification of toxicological properties of leachate may not be satisfactory. Due to the complex nature of leachate produced by solid wastes toxicity tests with leachates has not been fully standardized. Certain properties of organic compounds such as low solubility in water and volatilization have perhaps limited the interest in applications of toxicity test with such a complex mixture. Nevertheless, the application of toxicity tests to leachates with virtually unknown chemical composition is still continue and attempts are being made to improve and validate current procedures and methods. Currently, toxicological methods which employ aquatic or soil organisms can be directly, or with minor modifications, applied to leachates. Some authors have used composite parameters such as total suspended solids or COD to evaluate toxicity effects of leachate [6].

Acute toxicity test of landfill leachate from Sin Ginker landfill in Hong Kong [7] has been evaluated using *Sarotherodon mossambicus*, commonly known as tilapia, as the test organism. This leachate was strongly polluted with organic pollutant with total solid waste of 7700 (mg/L) and total Kjeldahl – N of 1053 (mg/L). Results of 96-hr toxicity test of tilapia in the leachate collected in July resulted to a LC50 of 12.3% v/v of the raw leachate. Similar assessment of an untreated landfill leachate with COD of 133 mg/L was conducted using freshwater arthropods resulted in 96 h LC50 of 57% v/v for *Asellus aquaticus,* and 5 v/v for *Gammarus pulex* [6]. Similar studies [8] **have** evaluated the toxicity of a landfill leachate in Okayama in western Japan using bioassays in the Japanese killifish Medaka *Oryzias latipes*. They showed that the leachate solution was lethal to larval and adult Medaka and caused inhibitory effect on hatchability of the embryos exposed to leachate solution. The LC50 values of the

Table 4: Lethal concentration (LC50) of Kermanshah MSW leachate and their lower and upper 95% confidence limits calculated using US-EPA analysis program.

	Exposure time (h)			
Oncorhynchus mykiss	24	48	72	96
Lower 95% confidence level	8.84	7.494	5.45	3.96
LC50	13.25	10.75	8.29	6.07
Upper 95% confidence level	47.52	21.98	17.01	10.79
	Exposure time (h)			
Gambosia affinis	24	48	72	96
Lower 95% confidence level	12.81	10.77	10.38	8.48
LC50	16.24	14.71	14.20	12.41
Upper 95% confidence level	37.51	30.16	28.08	26.04

leachate concentration with a COD of 79 mg/L were calculated as 53.0% for adults and 19.2% for larvae. The landfill leachates were shown to be highly toxic to both *C. dubia* and *S. capricornutum* with an EC50 of 10% and 15%, respectively.

Chemical analyses showed high concentrations of un-ionized ammonia and salts in the landfill leachates but low concentrations of heavy metals.

5 Conclusion

Results of over five decades of challenges in many different contexts including legislation, administration, science and technology in developed countries has ended in situations where in many countries waste management operations can be maintained by various degrees of landfilling, incineration with and without energy recovery, recycling and composting [9]. However, in most developing countries municipal solid waste management remains as a major problem. In these countries increasing portions of the population are now living in cities and poses serious challenges to the provision of municipal solid waste management services by the municipalities. However, these municipalities normally have little funds, are deficient in institutional organization and interest, have poor equipment for waste collection, and lack urban planning [10].

Table 5: Results of toxicity tests conducted by various aquatic organisms with MSW leachate with differing pollution load as indicated by COD, BOD or suspended solids.

Species	LC50 (v/v%)	COD (mg/L)	BOD (mg/L)	TSS (mg/L)	Reference
Sarotherodon mossambicus	12.3	-	-	7700	[7]
Oryzias latipes	53	79			[8]
Asellus aquaticus	57	133			[6]
Gammarus pulex	5	133			[6]
Embryos *Oryzias latipes* (L1)	1.6	740	374	74	[11]
Embryos *Oryzias latipes* (L5)	2	820	223	74	[11]
Embryos *Oryzias latipes* (L3)	2.2	1200	740	64	[11]
Embryos *Oryzias latipes* (L2)	2.4	5000	1700	496	[11]
Oncorhynchus mykiss	6	6250	2630	9398	Present study
Gambusia affinis	12	6250	2630	9398	Present study

Improper MSW management also directly affects environmental sanitation [12]. In developing countries lack of resources and legislation together with limitations caused by the market leaves very little choices for management options. These choices range from total lack of any operation for collection, transportation of solid wastes in the human settlements to improper collection, transportation and release into open dumps.

References

[1] Oman C B and Junestedt C, 2008. Chemical characterization of landfill leachates- 400 parameters and compounds. *Waste Manage*, 28: 776–794.
[2] Tsiridis V, Petala M, Samaras P, Hadjispyrou S, Sakellaropoulos G, Kungolos A, 2006. Interactive toxic effects of heavy metals and humic acides on Vibrio fischeri. *Ecotox Environ Saf*e, 63(1): 158-167.
[3] Loizidou M, Kapetanios E G, 1993. Effect of leachate from landfills on underground water quality. *Sci. Total Environ*, 128 (1): 69-81.

[4] Ding A, Zhang Z, F J, Cheng L, 2001. Biological of control of leachate from municipal landfills. *Chemosphere*, 44 (1): 1–8.
[5] Alavi M R, et al., 2008. Municipal solid waste management in Rasht city, Iran, doi: 10.1016/j. *Waste Manage*, 2008.02.029.
[6] Bloor M C and Banks, C J, 2006. Acute and sub-lethal toxicity of landfill leachate towards two aquatic macro- invertebrates: demonstrating the remediation potential of aerobic digestion. *Hydrobiologia*, 556: 387–397.
[7] Wong M H, 1989. Toxicity test of landfill leachate using *Sarotherodon mossambicus* (Freshwater Fish). *Ecotox Environ Safe*, 17: 149- 156.
[8] Osaki K, Kashiwada S, Tatarazako N and Ono Y, 2006. Toxicity testing of leachate from waste landfills using Medaka (*oryzias latipes*) for monitoring environmental safety. *Environ Monit Assess*, 117: 73–84.
[9] Kollikkathara N, Feng H and Stern E, 2008. A purview of waste management evolution special emphasis on USA doi: 10.1016/j. *Waste Manage*. 2008.06.
[10] Rotich K H, Yonsheng Z, Jun D, 2006. Municipal solid waste management challenges in developing countries- Kenyan study. *Waste Manage*, 26: 92-100.
[11] Kaur R, Buckley B, Park S S, Kim Y K, Cooper K R, 1996. Toxicity test of Nanji Island landfill (Seoul, Korea) leachate using Japanese Medaka (*Oryzias latipes*) embryo larval assay. *Bull. Environ. Contam Toxicol*. 57: 84-90.
[12] Mensah A, 2006. People and their waste in an emergency context. The case of Monrovia, Liberia. *Habitat Int*, 30 (4): 754–768.
[13] Standard Methods for the Examination of Water and Wastewater.1998. In: Clesceri LS, Greenberg AE, Eaton AD, editors. USA: *Water Environ* Federation.