January 2008 Volume 3 Issue 1

Environmental Science

Trade Science Inc.

An Indian Journal

Current Research Paper

ESAIJ, 3(1), 2008 [119-124]

Safety assessment of wastewater drained from landfill using medaka (*Oryzias latipes*) in aquarium system

Makiko Doi, Kazuhiko Fukatsu*

Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Tsukide, Kumamoto-862-8502, (JAPAN)

E-mail : fukatsu@pu-kumamoto.ac.jp

Received: 8th January, 2008 ; Accepted: 13th January, 2008

ABSTRACT

There are more than 10,000 different chemical substances in the environment, and many of them exist as a complex mixture of unknown substances in leachate from landfill, which can carry the chemicals into waterways. Assessing the risk that these potentially harmful chemicals pose to humans and wildlife is difficult. The aim of this study is to establish the use of aquarium-cultured medaka fish (*Oryzia latipes*) as a practical system for monitoring the safe of the treated water of leachate from landfill sites. Leachate that has been treated by desalination or activated-carbon filtration is thought to be of sufficient quality to support breeding populations of medaka, with no adverse reproductive consequences. We bred medaka in an aquarium containing water that had been treated by the leachate-treating equipment at a model landfill plant. We recorded and analyzed the survival rate, sex ratio, and reproduction stability (fecundity, fertility, hatchability, and deformity) in experimental and control aquarium-cultured medaka populations. Medaka grew and reproduced normally in the treated water. Based on our results, we proposed the establishment of a practical assay that uses aquarium-cultured medaka ecosystems and surrounding areas. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Landfill;
Leachate;
Medaka (*Oryzia latipes*);
Safety assessment;
Aqua culture.

INTRODUCTION

It is well known that various and harmful chemical substances are contained in the leachate from landfill sites. Moreover, endocrine disrupting chemicals such as phenols, dioxins and so on, have been detected in leachate[1-3]. Landfill management standards for the maintenance of water quality have been set out in order to protect environmental water quality at landfill sites; biochemical water quality monitoring of both the drainage from a landfill and the surrounding groundwater is required for this kind of management. However, biochemical monitoring cannot prevent contamination by unidentified or non-regulated substances; only substances for which harmful concentrations have been determined are regulated and required to be monitored biochemically. Furthermore, although biochemical analysis can supply detailed information on specific contaminants, it cannot describe the long-term influence that a collection of contaminants might have on an ecosystem. Biochemical techniques do not provide the practical information needed by local residents in order to realistically understand the risks associated with landfill sites. Accurate information concerning the safety of the drainage from a landfill would promote public understanding of the risks associated with a landfill site. To this end, it is necessary to establish a method of monitoring the long-term effects of water quality on an ecosystem. The water quality inspection guidelines (The OECD Guidelines for the Testing of Chemicals[4]), established by the Organization for Economic Co-operation and Development (OECD), use biological indicators, such as seaweed, daphnia, or fish, as a way of evaluating water quality with respect to ecosystem preservation: this represents an advanced approach to water quality monitoring in that it takes the soundness of an entire ecosystem into consideration as opposed to the conventional monitoring, which is performed only in relation to human health concerns.

This investigation constitutes basic reserach for establishing the use of aquarium-cultured medaka fish (*Oryzias latipes*) as a practical system for monitoring the environmental safety of the treated water from landfill sites. Previously, we reported the results of short-term exposure tests in which we examined the physiological responses for medaka to the leachate and the treated water from landfill sites[5]. This paper describes an investigation of the aquarium environment and the reproduction stability of medaka populations that were raised in aquaria containing treated water from a landfill site. Based on our findings, we propose the establishment of a practical assay that uses aquarium-cultured medaka to assess the safety of the drainage from landfill sites.

EXPERIMENTAL

Fish (medaka) and sample water

Mature medaka was purchased from a local fish farm in Kumamoto, Japan and a breeding stock of medaka was maintained in a glass tank in our laboratory. During breeding, the fish were placed under 16 : 8-hrs light : dark photoperiod at 26±1°C. The medaka were fed *Artemia nauplin* (< 24hrs after hatching) as well as a commercial diet (Kyorin, Japan), three times in a day.

TABLE 1 : Composition of waste in model landfill

Sample waste	Weight (t)	Weight ratio (%)
Bottom ash	72.3	41.5
Crushed waste	72.3	41.5
Sludge	3.0	1.7
Cover soil	26.0	15.3
Total	173.6	100.0
Volume		$117\ m^3$
Density		$1.48\ t\ m^{-3}$

TABLE 2: Chemical analysis of wastewater drainaged from model landfill site

Parameter	(Unit)	Leachte	AC filtered	Desalinized
pH	-	12.0	7.6	7.3
SS		7.5	-	<0.5
TOC		1,360	37.2	7.0
BOD		2,250	2.8	0.6
COD		918	25.4	1.6
T-N		231	-	1.1
Pb	$(mg\ L^{-1})$	0.042	-	<0.005
Cd		0.001	-	<0.001
Hg		<0.0005	-	<0.0005
As		<0.005	-	<0.005
Na		5,670	-	86.5
K		4,670	-	56.4
Ca		4,360	6.7	0.3
Cl		17,800	4,720	17.8
Benzophenone		0.11		<0.01
Bisphenol A		140		0.13
Nonylphenol	$(\mu g\ L^{-1})$	24		3.6
4-t-Butyl phenol		<0.01		<0.01
Butylbenzyl phthalate		<0.2		<0.2
Dibutyl phthalate		<0.2		<0.2

The leachate from a large model landfill plant was treated in the leachate-treating equipment obtained for this model landfill plant as an addition. This model landfill contained incineration ashes, shredder residue, and sludge, which are representative of common landfill waste in Japan. The mixture ratio of the waste components is shown in TABLE 1.

The leachate-treating equipment consists of calcium removal, biological treatment, chemical clarification, activated carbon filtration, and desalinization devices [6]. Desalinization is accomplished by an electric dialysis as a final treatment. Sample water were desalinized water (the product of the final treating step) and activated carbon-filtered water (the product of the treating step just before desalination). Tap water, which was filtered with activated carbon, was used as control water. TABLE 2 shows the analysis data of leachate and treated water from this model landfill site. For each sample water, 60 juvenile medaka were bred for 3 months in a 50 L aquarium containing the sample water.

Acute toxicity test

The acute toxicity test was performed for the adult, juvenile, and embryo. In this investigation, the purchased adult medaka was acclimatized for a week in our laboratory, and the population of 5% mortality or less was used. The 30 embryos employed for each treatment were separated into three groups of 10 embryos each for testing in triplicate. The control embryos were exposed to control water. The embryos in each group were placed in the 24-well microplate, each containing 3 ml of the test solution, and then incubated under 16 : 8-hrs light : dark photoperiod cycle at 24±1°C until the last embryos hatched. The test solution in the wells was changed every 24 hours. The developing embryos were observed daily under a stereoscopic microscope, and dead embryos were removed daily.

The 20 juvenile from each treatment were placed in glass beakers, each containing 1000 ml of test solution at 25±1°C, and were not fed during the toxicity tests. The exposure water in the beakers was not changed during the test period. The controls in the adult test were conducted in 10% artificial seawater. In the adult test, the conditions were the same as those in the juvenile test, expect that the number of fish in each group was 15 individuals and they were fed one time daily, and aeration was carried out under test in order to prevent a reduction in dissolved oxygen. These tests were conducted in concordance with the 16: 8-hrs light : dark photoperiod cycle.

Life cycle assessment

For each sample waters, 60 juvenile medaka were bred for 3 months in a 50L aquarium containing the sample water. Groups of two male and one female adult medaka were selected from the sample water cultures and maintained in a glass beaker containing 1000ml of dechlorinated tap water at 26±1°C for 7 days with a photoperiod of 16 hrs light : 8hrs dark; the breeding water was exchanged daily. The fish were fed *Artemia naupline* and a commercial diet at three times in a day. The number of eggs spawned in each group was counted daily for 7 days, and the ratio of fertilized eggs was calculated. All surviving medaka were paired as male and female, and medaka pairs were exposed to control water. The embryos spawned from each female were collected, counted, and checked for fertilization and development under a stereoscopic microscope. Each embryo was retained in control water until it hatched, and hatchability and malformation were determined. At the end of exposure, livers and gonads were sampled, and the weight and the length of each fish were measured. The gonad somatic (GSI,%) and the hepatoso matic index (HIS, %) were also calculated as a ratio of gonad and liver weight to body weight. Also, hepatic vitellogenin (VTG) levels of medaka were measured by using a Medaka Vitellogenin ELISA Kit[7] purchased from Transgenic Inc., Kumamoto, Japan that uses enzyme-linked immunosorbent assay.

RESULTS AND DISCUSSION

Acute toxicity test in short-term exposure

We previously reported that the survival rate of medaka was remarkably low in untreated leachte and juvenile was easily affected by toxic chemicals because juvenile was very small and their defense function was weaker than that of adult fish[8]. As shown in TABLE 2, the leachate from a large model landfill plant contains hi-dense and various chemical substances. Although the leachate is the quality of high salt and alkalinity due to incineration ashes, the desalinized water is colorless transparence, and the content of chemical substances is reduced remarkably by the treatment of leachate-treating equipment. The chemical analysis data reveal that the desalinized water is chemically similar to tap water.

TABLE 3 shows the result of the acute toxicity test in a short-term exposure. It was suggested that the desalinized water did not affect the growth stages of medaka in the short-term exposure, because the sur-

TABLE 3: Survival rate in growth stages of medaka in short-term exposure

Exposure water	Adult n Survival rate (%)	Juvenile n Survival rate (%)	Egg n Hatchability (%)
Contraol	45, 93.3 (84.4~102.2)	60, 81.7 (71.9~91.5)	90, 100
Desalinized	45, 97.8 (93.5~102.1)	60, 80.1 (67.8~92.4)	90, 100

Values in parenthesis is mean 95% confidence limits.

TABLE 4: Reproduction test of medaka in short-term (7 days) exposure

Exposure water	Number of spawn	Fertilization rate (%)	Hatchability (%)
Control	104.8±16.9	98.3±1.8	94.5±0.9
Desalinized	110.4±11.2	98.0±1.8	92.0±6.0
Control	87.8±29.1	97.6±4.0	93.0±3.3
AC filtered	96.4±22.5	97.6±4.4	95.7±4.3

Values are mean 95% confidence limits

TABLE 5: Sex ratio and survival rate of medaka grown in long-term exposure

Exposure water	Survived number	Sex		Survival rate (%)
		Male	Female	
Control	30	21	9	50
Desalinized	42	22	20	70
Control	36	23	13	60
AC Filtered	45	20	25	75

$P > 0.05$, 2×2 test

vival rate and hatchability in the exposure of desalinized water were nearly equal to that of control water. As shown in TABLE 4, the fecundity of paired medaka in a long-term exposure was not affected by control and desalinized water. The breeding pairs in all exposure groups and in the control spawned every day, and their mean fertility was maintained at more than 90% . The mean time to hatch was about 10 days (not shown in TABLE). There was no significance in the fecundity, fertility, hatchability and time to hatch among all control and desalinized water exposure groups. Acute toxicity was not observed on each growth stages by the exposure of this test. Moreover, there is no significant difference in hepatic vitellogenin (VTG) production though the data was not shown.

Survival rate

All fish displayed normal growth and reproduction in the aquaria filled with treated water, just as in the comparable aquarium filled with control water. The survival rates of medaka grown in the desalinized water and the activated carbon-filtrated water were higher than those in control water, as shown in TABLE 5. Fish eggs and juveniles are reported to have a resistance to stress[9,10], but not enough is known about the stress response mechanisms in fish to allow a reasonable investigation of the effects of stress on fish health [11]. The specific properties of tap water, e.g., pH, heavy metal and mineral concentrations, and the presence of chlorine or organic matter, can impair the health of fish and reduce the probability of survival for juveniles and eggs.

Also, excessively high or low concentrations of mineral salts in control water can reduce the survival rate for juveniles and eggs[12]. Both the breeding and the survival rate of fish in tap water were lower than those of fish in the treated water. Generally, the lack of mineral and organic substances in tap water influence the health of fish, and lower the survival rate of juveniles and eggs. As one example, it is has been found that the survival rate is raised by adding leachate to tap water in which the survival rate is low[12]. Thus, the detection of a toxic substance in water does not necessarily indicate that the water will have a negative impact on organisms, nor can it be assumed that water is safe because no known toxic substance was detectable. The survival rate of juveniles and eggs is perhaps the most demonstrative estimate of stable water quality.

Sex ratio

While the sex ratio is 1 : 1 in the nature, there was a tendency for the proportion of males to increase in the medaka population bred in control water, based on two examinations as shown in TABLE 5. But there was no significant difference between the sex ratio of the medaka grown in tap water and that of the medaka grown in either of treated waters ($p>0.05$, 2×2 test). It has been reported in fish, as well as in some invertebrate, that a chemical substance can change the sexual characteristics of an individual[1]. However, the increase in the proportion of males in this investigation seems to be because the female were weak in control water used in this examination. It is difficult to imagine the sex reversal happened in this investigation[13]. Activated carbon-filtrated tap water is usually used as a control water as well as this experiment, as a tap water quality is different according to the difference between sauce water quality and treatment process in the region [14]. That is to say, the sensitivity of the female will be strong for the water used in this investigation. Though we are observing this phenomenon, its reason is not yet clear. Therefore, it is necessary to carry out further examination.

Reproduction stability

TABLE 6 shows the reproduction stability of the medaka. The fertility and hatchability were normal for

TABLE 6: Reproduction stability of medaka in long-term exposure

Exposure water	Eggs in one spawning	Spawning days in a week	Fertilization rate(%)	Survival rate (%)
Control	20±3.2	6.3	86±10	81±14
Desalinized	12±3.2	6.5	86±6	91±4
Control	16±3.2	6.8	90±10	92±8
AC filtered	16±2.2	5.4	88±4	93±3

TABLE 7: Dissection examination of medaka grown in long term exposure

Sex	Exposure water	Body weight (g)	HSI (%)	GSI (%)
Male	Control	0.28±0.03	1.3±0.5	0.7±0.3
	AC filtered	0.28±0.03	1.4±0.5	0.7±0.3
	Desalinized	0.41±0.08	1.6±0.5	0.6±0.3
Female	Control	0.32±0.06	2.3±0.8	3.8±1.7
	AC filtered	0.31±0.04	2.8±0.8	6.0±0.2*
	Desalinized	0.44±0.07	3.2±1.2	7.1±2.0*

*Dunnet post hock test, $p<0.05$

80% or greater of the spawned eggs, and there were no malformations. Unusual embryo and juvenile body shapes can occur naturally, with a rate of up to several percent when mating is between related individuals.

No malformation was observed among juveniles in this investigation, even though juveniles frequently show malformations among fish bred in a laboratory. The two primary causes of deformation in laboratory-bred fish are thought to be either a deterioration in the quality of an order female's eggs, or genetic defects in a specific medaka lineage. Before using a line of medaka for monitoring, its rate of malformations should be evaluated under control conditions. Spawning, however, did not occur on every one of the 7 days, while in the previous short-term exposure test, the medaka spawned daily. This is because only medaka that had bee checked in advance for the ability to spawn daily were used to evaluate reproduction before and after a short-term exposure, whereas all the cultured fish were used for the repuroductive testing of medaka after a long-term exposure, since the purpose of this experiment was to evaluate the reproduction stability of individuals grown in sample water.

Physiological examination

All individuals were dissected, after the reproduction stability test ended in the long-term exposure. Its results are shown in TABLE 7.

Although the body weight (BW) and the hepatosomatic index (HIS) of the fish did not differ significantly in the treated water, a female medaka grown in control water showed a significantly lower gonad somatic (GSI). The grand cell in the female, which should have ripened in the gonad, was not observed with the binocular microscopy. Although we observed a significantly lower GSI in female bred in control water, this is probably the result of different growth and maturation rates due to the variability in the quality of control water. Because the reproduction stability was examined under conditions of variable tap water quality, the differences in the number of eggs or the frequency of spawning would be expected. Even when medaka are bred in the same tank, body sizes and maturity peaks can be different. The unpredictability in the time of maturation and the decline in breeding ability complicate the studies of reproduction stability.

In this investigation, we used a pair testing to assess the reproduction stability but this method is not an ideal method for testing the reproduction stability of aquarium-cultured medaka, because the numbers of male and female fish are not equal. In addition, females often have preferences among male fish, and a pair test would not take this into account. A more useful assessment method of the reproduction stability would be to gather eggs directly from the aquarium. It would also be important to check the age at which spawning begins, and the number of spawning female.

CONCLUSIONS

In order to establish a practical monitoring system for the breeding of medaka in drainage from landfill site, we have carried out a short-term and a long-term exposure tests to medaka using the treated water from a large model landfill site. There was not a difference in the survival rate between desalinized water and control water in a short-term exposure, but in a long-term exposure, the survival rate lowered in control water; and as for the sex ratio, the males tended to abound in control water. In physiological examination, although malformation among juveniles was not observed in either water, a female medaka bred in control water showed a significantly lower gonad somatic (GSI). But in the reproduction stability test, the egg-laying condition was normal. The reduction of the gonad in control water seemed to be faster than in the treated water because the egg-laying peak has expired before the dissection. It was proven that the treated water used in this investigation was of a quality in which the breeding of medaka was possible and the adverse effect did not cause the reproduction. Since there was no difference between activated carbon-filtered water and desalinized water in the safety assessment in this investigation, it would seem that advanced treatment such as electric dialysis is not always necessary in the treatment of leachate. These findings in this investigation suggest that an aquarium using medaka is effective to evaluate the safety of treated landfill drainage water.

REFERENCES

[1] T.Colborn, F.S.vom Saal, A.M.Soto; Enviro.Health Persp., **101**, 378 **(1993)**.

[2] Y.J Kim, M.Ohsako, D.H.Lee; J.Jpn.Soc.Water Enviro., **10**, 214 **(1999)**.

[3] 3.H.Shiraishi, O.Nakasugi, S.Hashimoto, T.Yamamoto, A.Yasuhara, K.Yasuda; Waste Management Res., **10**, 214 (1999).

[4] http://www.oecd.org./department/0,3355,en_2649_34377_1_1_1_1_1,00.html;5th Jan, **(2008)**.

[5] M.Tanaka, K.Fukatsu, R.Shinohara, M.Hanashima, S.Higuchi, K.Tomoda; 'A Simple EDCs monitoring system of a landfill site by *Oryzias latipes* for the coexistence with the local community', Paper Presented at the Second Asian Pacific Landfill Symposium, Seoul, Korea, 25-27 Sept, **(2002)**.

[6] T.Akihiro, U.Kenichi; 'Treatment of landfill leachte with RO', Paper Presented at the First Asian Pacific Landfill Symposium, Fukuoka, Japan, 11-13 Oct., **(2000)**.

[7] http://www.transgenic.co.jp/; 5th January, **(2008)**.

[8] M.Doi, K.Fukatsu, R.Shinohara, M.Hanashima, S.Higuchi, K.Tomoda; J.Jpn. Soc.Waste Manage. Experts, **15**, 294 **(2004)**.

[9] T.Yano; Gekkan Kaiyo Extra, **14**, 124 **(1998)**.

[10] Y.Suzuki; Nippon Suisan Gakkaishi, **66**, 372 **(2000)**.

[11] Y.Suzuki, S.Tasumi; Gekkan Kaiyo Extra, **18**, 125 **(1999)**.

[12] M.Tanaka, T.Takahashi, **(2002b)**: 'Softening Disease of Medaka (*Oryzias latipes*) egg by Exposure of Tap Water', Paper Presented at the 13th Conference of the Japan Society of Waste Management Experts, Kyoto, Japan, 28-30 Oct., **(2002)**.

[13] U.Kasuga; Bull.Japanese Soc.Scientific Fisheries, **46**, 1073 **(1980)**.

[14] M.Doi, Y.Takamura, K.Fukatsu, M.Kono; J.Japan Soc.Water Environment, **27**, 343 **(2004)**.