

Effect of Nickel on Embryo Development and Expression of Metallothionein Gene in the Sea Urchin (*Hemicentrotus pulcherrimus*)

Hwang, Un Ki

West Sea Fisheries Research Institute, Marine Ecological Risk Assessment Center

Park, Jong Soo

West Sea Fisheries Research Institute, Marine Ecological Risk Assessment Center

Kwon, Jung No

West Sea Fisheries Research Institute, Marine Ecological Risk Assessment Center

Heo, Seung

West Sea Fisheries Research Institute, Marine Ecological Risk Assessment Center

他

<https://doi.org/10.5109/22062>

出版情報：九州大学大学院農学研究院紀要. 57 (1), pp.145-149, 2012-02. Faculty of Agriculture, Kyushu University

バージョン：

権利関係：

Effect of Nickel on Embryo Development and Expression of Metallothionein Gene in the Sea Urchin (*Hemicentrotus pulcherrimus*)

Un Ki HWANG¹, Jong Soo PARK¹, Jung No KWON¹, Seung HEO¹,
Yuji OSHIMA² and Han Seung KANG^{1*}

Division of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences,
Kyushu University, Hakozaki 6–10–1, Higashi-ku, Fukuoka 812–8581, Japan
(Received October 31, 2011 and accepted November 9, 2011)

Sea urchin embryo has been used to monitor pollutants in marine environments. Nickel (Nickel chloride, Ni), as a heavy metal, is a chemical element with the chemical formula $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. It may cause harmful effects on the central nervous system and growth. Metallothionein (MT) is a metal binding protein and it play a regulatory role in the homeostasis and detoxification of heavy metals. In this study, we examined the gametotoxic and embryotoxic effects of Ni at various concentrations (0, 10, 25, 50, 100, 500 ppb) in the sea urchin *Hemicentrotus pulcherrimus*. Spawning was induced by injecting 1 ml of 0.5 M KCl into coelomic cavity. Experiment was begun within 30 min the collection of both gametes. The fertilization and embryo development rates test were performed for 10 min and 64 h after fertilization, respectively. Results of this study, the fertilization rates in the control condition (not including Ni) and experimental group were not significantly changed. However, the embryo development rates were significantly decreased with concentration dependent manner. Especially, 50 ppb Ni treatment was significantly lower than vehicle control at 64 h onward. The normal embryogenesis rate was significantly inhibited in exposed to Ni (EC_{50} =34.19 ppb, 95% CI=29.56–38.09 ppb). The NOEC and LOEC of normal embryogenesis rate were <10 ppb and 25 ppb, respectively. The expression of MT mRNA gene was significantly increased with concentration dependent manner. These results suggest that the early embryo stages of *H. pulcherrimus* have toxic effect at greater than 25 ppb of Ni concentration and MT mRNA gene may be used as a biomarker gene for risk assessment on contamination of Ni.

Key words: 95% CI, EC_{50} , *Hemicentrotus pulcherrimus*, Metallothionein, Nickel

INTRODUCTION

Pollution associated with urbanization and industrialization endanger marine ecosystems. Heavy metals such as nickel, cadmium, zinc, lithium, and the major constituents of industrial wastes, they affect the marine ecosystem (Gopalakrishnan *et al.*, 2008). These heavy metals have embryotoxic/teratogenic effects by directly or indirectly (Calevro *et al.*, 1998). Nickel, as a heavy metal, the chemical symbol Ni and atomic number 28, should mainly target on active sites of enzymes. In embryos testing of Ni, a number of studies showed that Ni affect toxicity (Léonard *et al.*, 1981; Lu *et al.*, 1979; Luo *et al.*, 1993; Saillenfait *et al.*, 1991; 1993; Sunderman *et al.*, 1980).

Metallothionein (MT) is a low molecular weight cysteine-rich metal-binding proteins, is present in many phylogenetically diverse organisms, and is induced by many chemicals, including metals, glutathione depletors, and hydrogen peroxide (Dalton *et al.*, 1994; Klaassen *et al.*, 1999; Sato *et al.*, 1995; Zhu and Thiele, 1996). In previous reported that MT proteins play a regulatory role in the homeostasis of zinc and copper (Cosson,

1994; Cousins, 1985; Fukushima *et al.*, 1988; Olsson *et al.*, 1989), and detoxification of cadmium and mercury (Debec *et al.*, 1985; Din and Frazier, 1985; Kay *et al.*, 1987; Kito *et al.*, 1982;). Also, MT was decreased the chemical carcinogenesis (Dalal and Bhattacharya, 1991; Takaishi *et al.*, 2009).

Sea urchin embryo larval development has been studied and used to monitor pollutants in marine environments over the last several decades (Flammang *et al.*, 1997; Kobayashi, 1971; 1994; Okubo and Okubo, 1962; Tabata, 1956). In the two main life stages, the embryo larval and adult stages were generally studied and used in testing (Bielmyer *et al.*, 2005). In particular, the early life stages of sea urchins have been shown to be sensitive to metals (Kobayashi, 1973; 1980; Phillips *et al.*, 2003).

The present study was conducted to evaluate the acute toxicities of Ni in sea urchin (*H. pulcherrimus*) embryo and to predict these toxicities in the marine environment.

MATERIALS AND METHODS

Sample and collection

The organisms that were used for this study are sea urchins (*H. pulcherrimus*) which belong to the marine invertebrate classification Echinodermata that inhabit the rock areas of the entire Korean coast. The developed *H. pulcherrimus* can be easily collected at any time, as they have different spawning periods that depend on water temperature prevalent across the intertidal zones.

¹ West Sea Fisheries Research Institute, Marine Ecological Risk Assessment Center, Eulwang-dong, Jung-gu, Incheon 400–420, Korea

² Division of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Hakozaki 6–10–1, Higashi-ku, Fukuoka 812–8581, Japan

* Corresponding author (E-mail: kanghs@nfrdi.go.kr)

Test organisms were collected from the intertidal zone of the Buan Fish Hatchery in Gyeokpo-ri, Byeonsan-myeon, Buan-gun, Jeollabuk-do between March and April 2011, the season for the main spawning period. The collected sea urchins were kept in a laboratory with a water temperature of about $9 \pm 1^\circ\text{C}$ for over a week before the test.

Prepared sperm and ovum

Sterilized sea water was used to remove the protozoa or other foreign matters that might be on the sea urchin surface. Eighteen of this species, with 3.5 cm or more diameters, were used for spawning. The natural sea water was filtered (using a membrane filter with a pore size of $0.45 \mu\text{m}$) and sterilized before it was used in the test. A 100 ml beaker was filled with the filtered natural sea water to sufficiently soak the genital pore, and 1 ml 0.5 M KCl solution was injected in the coelomic cavity. Gametes were obtained from the 30 min spawning. The sperm solution was washed once, and the ovum solution was washed thrice before they were used for the test (Fujisawa, 1989).

Nickel treatment and fertile eggs–embryos testing

Nickel (Nickel chloride, Ni, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, CAS No.

7718–54–9) was obtained from Shamchun Chemicals (Korea) and dissolved in sterilized sea water. Fertilized eggs and embryos were obtained from at least three different male/female pairs for each bioassay. Sperms were exposed to varying concentration of nickel (0, 10, 25, 50, 100 and 500 ppb in sterilized sea water) for 30 min; after which, the ova were injected. 10 min later, the fertilization rates were identified by checking whether the fertilization membrane was formed. They were transferred in the cap tube, fixed with formalin solution (3%), and observed using an optical microscope (Fig. 1). To examine the normal embryogenesis rate, 64 h after adding the ova, the specimens were fixed with formalin solution (3%); they were then observed using an optical microscope that would help classify them into normal and abnormal pluteus, that is, small in size and deformed (Pagano *et al.*, 1985a; b) (Fig. 1). The test medium was not changed.

Experiment was replicated three times, while 100 or more embryos exposed to the test solution were also repeatedly counted three times to determine the ratio of normal embryos to fixed embryos in percentage. To assess the toxicity of the Ni using the results, the probit analysis method was used to analyze the 50% effective concentration (EC_{50}) and the 95% confidence limit (95%

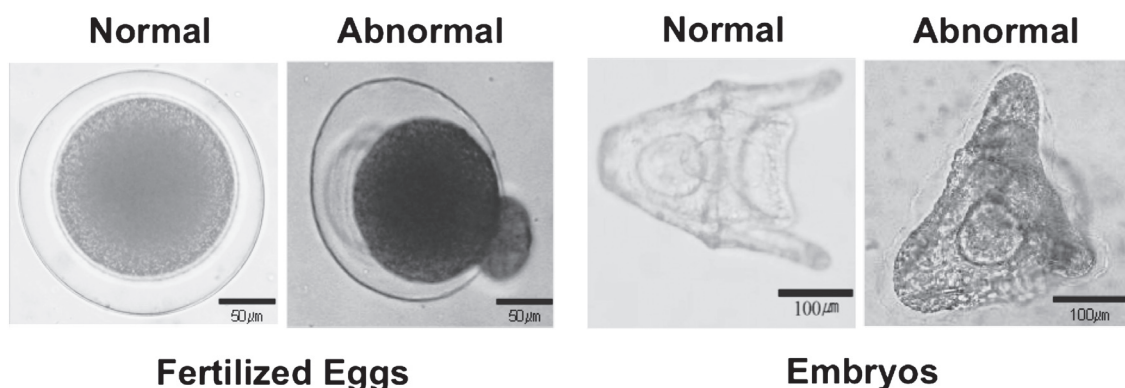


Fig. 1. Normal and abnormal forms in fertilized eggs and embryos of sea urchin (*H. pulcherrimus*).

Table 1. Experimental culture conditions using the sea urchin (*H. pulcherrimus*)

Test parameters	Conditions
Culture type	Static non-renewal 10 min – 64 h toxicity test
Photoperiod	Ambient light condition and 8L:16D periods
Temperature	$16 \pm 0.5^\circ\text{C}$
pH	7.8 – 8.2
Salinity	32 ± 1.0
Chamber volume	80 ml glass
Solution	Filtered ($0.4 \mu\text{m}$) and sterilized seawater
Solution exchange	None
Experiment period	10 min – 64 h
Investigation item	Fertilization, larval development rates
Acceptability criterion	> 90% fertilized eggs and pluteus larvae at control

CI) of the fertilization and normal embryogenesis rate. In addition, no observed effective concentration (NOEC) and lowest observed effective concentration (LOEC) were analyzed using the Dunnett's test.

The embryos were cultured in an incubator (LMI-3004PL, DAIHAN Lab Tech, Korea) at $16 \pm 0.5^\circ\text{C}$ for 64 h. The pH of the culture fluid was kept constant at 7.8–8.2. Via the preliminary test for optimal sperm addition, sperms were diluted by 2,000–2,500 times, and 1,500–2,000 fertilized eggs were added to 1 ml of test water used for the culturing process based on the conditions in Table 1.

Total RNA extraction

Total RNA extraction was carried out with RNAiso Plus (TaKaRa Shuzo Co. Ltd, Japan). Briefly, sample of 1 ml culture embryos were homogenized in 1 ml of RNAiso Plus reagent. Total RNA was separated from DNA and proteins by adding 0.2 ml chloroform, and was precipitated using isopropanol of 1.0 x the volume of the upper layer. The precipitate was washed twice in 75% ethanol, air-dried for 3 min and re-diluted in diethylpycocarbonate (DEPC)-treated distilled water (DW). The amount and purity of extracted RNA were quantitated by Nanovue (SE-75184, Healthcare Bio Sciences, Korea).

Reverse transcription (RT)

For reverse transcription (RT), the AccuPower RT Premix kit (Bioneer Co. Ltd, Daejeon, Korea) was used. The RT Premix tube was prepared for each sample and add to 20 μl DEPC-DW, 50 pmol oligo(dT) primer and 0.3 μg total RNA from each sample. RT was carried out in a TProfessional Thermocycler (Biometra GmbH, Germany) using a program with the following parameters: 42°C for 60 min, 99°C for 5 min, and then quenched at 4°C . After the reaction was completed, samples were stored at -20°C until the PCR.

Polymerase chain reaction (PCR)

Sequences of oligonucleotide primers for detection of MT and Spz12-1 in sea urchin were obtained from GenBank (MT, Accession: NM214577; Spz12-1, Accession: U19831) and synthesized by Bioneer Co. (Korea). The primer sequences are: MT forward 5-CAAGGATGGAACCTGCTGTG-3; MT reverse 5-GCAGATGCGCTGAGTGACTT-3; SpZ12-1 forward 5-AAAATGACACAGGCGATGGA-3 SpZ12-1 reverse 5-TAATGTTTCTCGCTCCCCCT-3. Spz12-1 mRNA expression was employed as an internal positive control and was detected in all the samples studied, thus assuring the integrity of the RNA extraction and RT-PCR processes. PCR was performed following the protocol suggested by the manufacturer. The reaction was carried out in the AccuPower HotStart PCR Premix kit (Bioneer Co. Ltd, Korea). After an initial denaturation step for 5 min at 95°C , the amplification consisted of a total 28 cycles for MT and 26 cycles for SpZ12-1 at 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. The reaction was terminated at 72°C for 5 min and was quenched at 4°C . 2% agarose gel (SeKem, USA) electrophoresis was

carried out in 0.5 x TBE.

Statistical Analysis

Data were analyzed by one-way ANOVA (Fisher PLSD test). Fisher's test was also used to examine the significance of correlation coefficients. Significance was accepted at $*P < 0.05$, $**P < 0.01$.

RESULTS

When exposed to Ni, fertilized eggs and embryos showed developmental abnormalities including unfertilized eggs, rupture of fertilized eggs and deformed embryos (Fig. 1). Following the Ni treatment the fertilization rates were not changed (Fig. 2) and the rates of normal embryogenesis were gradually decreased in a concentration dependent manner (Fig. 3). The normal embryogenesis rates following 50 ppb Ni treatment was significantly lower than vehicle control at 64 h onward (Fig. 3).

Based on the test results determined from the effects of Ni treatment on the fertilization and normal embryogenesis rate of *H. pulcherrimus*, EC_{50} and 95% CI were calculated using the probit analysis method, and NOEC and LOEC were calculated using the Dunnett's test (Table 2). The EC_{50} of the rate of normal embryogenesis was 34.19 ppb. The 95% CI of EC_{50} was 29.56–38.09 ppb for the normal embryogenesis rate. The NOEC and LOEC of normal embryogenesis rate were < 10 ppb and 25 ppb, respectively (Table 2).

The effect of Ni on MT mRNA expression is shown in Fig. 4. Ni induced expression of MT mRNA significantly in *H. pulcherrimus*.

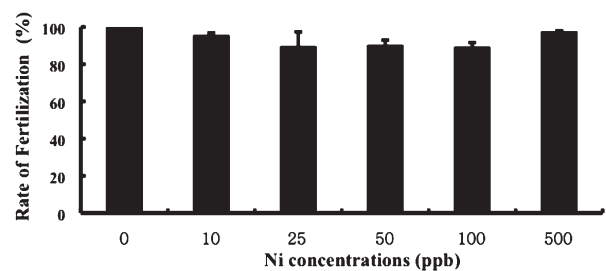


Fig. 2. Fertilization rates of sea urchin (*H. pulcherrimus*) exposed to Ni.

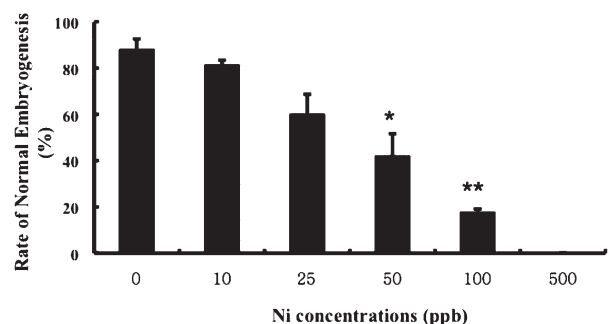


Fig. 3. Normal embryogenesis rates of sea urchin (*H. pulcherrimus*) embryos exposed to Ni. * Significant different from vehicle control by Fisher's exact test ($P < 0.05$), $**P < 0.01$.

Table 2. Toxicological estimation using the form of normal embryos in the sea urchin (*H. pulcherrimus*) exposed to Ni. EC₅₀: 50% effective concentration, 95% CI: 95% confidence limit NOEC: No observed effective concentration, LOEC: Lowest observed effective concentration

Items	Toxicity (End-points)	Ni (ppb)
EC ₅₀	Normal embryos	34.19
95% CI	Normal embryos	29.56–38.09
NOEC	Normal embryos	<10
LOEC	Normal embryos	25

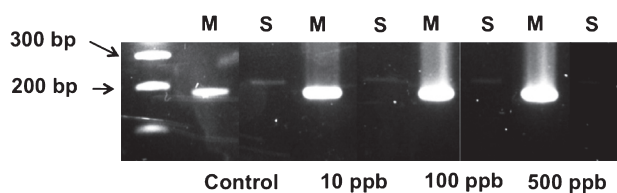


Fig. 4. Expression of MT and Spz12-1 mRNA genes in embryos exposed to Ni by RT-PCR. M: Metallothionein, S: Spz12-1.

DISCUSSION

The sea urchin embryo is an intact developing system, which undergoes events comparable to those of other vertebrates, including mammals. Sea urchin embryo normal embryogenesis and teratogenesis assay is useful because they can rapidly provide information on developmental toxicants.

Ni concentration in seawater generally range from 0.2 to 130 $\mu\text{g/l}$ (DETR, 1998). In exposed to Ni, the normal embryos of *H. pulcherrimus* were decreased with concentration dependent manner. At 50 ppb Ni, normal embryo was significantly decreased compared with vehicle control at 64 h onward. Following Ni treatment, embryos showed developmental abnormalities including unfertilized eggs, rupture of fertilized eggs and deformed embryos. The incidence of developmental abnormalities increased with increasing concentration of Ni. Saillenfait *et al.* (1993) reported that Ni caused growth retardation and brain and caudal abnormalities in rat embryo. Also, in rat embryos, Ni reduced caudal neural tube, branchial arches and dilated optic vesicles (Saillenfait *et al.*, 1991). In *Hydroides elegans*, Ni decreased rate of fertilization and delayed or blocked the first mitotic divisions and altered early embryonic development when the sperm and eggs were treated with Ni (Gopalakrishnan *et al.*, 2008). The NOEC and LOEC of Ni for normal embryogenesis were each <10 ppb and 25 ppb at 64 h in *H. pulcherrimus*. In the previous studies, Ni has been reported to have toxic effects in sea urchin (*Diadema antillarum*) and showed the 40 h EC₅₀ value was 15 $\mu\text{g/l}$ (Bielmyer *et al.*, 2005). Our results, in *H. pulcherrimus*, 64 h EC₅₀ value was 34.19 ppb, which is higher than *D. antillarum*. The differences in EC₅₀ values might be due to the different species and different exposure times in

the experiments. Taken together, we suggest that the safe guideline for Ni in marine environment should be below the 25 ppb for embryonic survival of *H. pulcherrimus*.

The induction of MT gene by heavy metals was demonstrated in sea urchin (*Paracentrotus lividus*) by Russo *et al.* (2003). MT gene expression showed an up-regulation by cadmium (Russo *et al.*, 2003). In rat kidney cells, MT was up-regulated and concentration dependent by Ni treatment (Lee, 2006). In animal cells, MT gene was rapid induction by exposed to heavy metal ions (Hamer, 1986; Karin *et al.*, 1987). In our result, Ni induced expression of MT mRNA gene significantly in *H. pulcherrimus*. These results suggest that MT mRNA gene may be used as biomarker gene for risk assessment on contamination of Ni.

REFERENCES

- Bielmyer, G. K., K. V. Brix, T. R. Capo and M. Grosell 2005 The effects of metals on embryo-larval and adult life stages of the sea urchin, *Diadema antillarum*. *Aquatic Toxicology*, **74**: 254–263
- Calevro, F., S. Campani, M. Raggianti, S. Bucci and G. Mancino 1998 Test of toxicity and teratogenicity in biphasic vertebrates treated with heavy metals (Cr³⁺, Al³⁺, Cd²⁺). *Chemosphere*, **37**: 3011–3017
- Cosson, R. P. 1994 Heavy metals intracellular balance and relationships with metallothionein induction in the liver of carp after contamination by silver, cadmium and mercury following or not pretreatment by zinc. *Biometals*, **7**: 9–19
- Cousins, R. J. 1985 Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol. Rev.*, **65**: 238–309
- Dalal, R. and S. Bhattacharya 1991 Effects of chronic non-lethal doses of non-metals and metals on hepatic metallothionein in *Channa punctatus* (Bloch). *Indian J. Exp. Biol.*, **29**: 693–694
- Dalton, T., R. D. Palmiter and G. K. Andrews 1994 Transcriptional induction of the mouse metallothionein-I gene in hydrogen peroxide-treated Hepa cells involves a composite major late transcription factor/antioxidant response element and metal response promoter elements. *Nucl. Acids Res.*, **22**: 5016–5023
- Debec, A., R. Mokdad and M. Wegnez 1985 Metallothioneins and resistance to cadmium poisoning in *Drosophila* cells. *Biochem. Biophys. Res. Commun.*, **127**: 143–152
- Department of the Environment, Transport and the Regions 1998 *Digest of Environmental Statistics No. 20 1998*. The Stationery Office, London (England)
- Din, W. S. and J. M. Frazier 1985 Protective effect of metallothionein on cadmium toxicity in isolated rat hepatocytes. *Biochem. J.*, **230**: 395–402
- Flammang, P., M. Warnau, A. Temara, D. J. W. Lane and M. Jangoux 1997 Heavy metals in *Diadema setosum* (Echinodermata Echinoidea) from Singapore coral reefs. *J. Sea Res.*, **38**: 35–45
- Fujisawa, H. 1989 Differences in temperature dependence of early development of sea urchins with different growing season. *Ref. Biol.*, **176**: 96–102
- Fukushima, T., Y. Ijima and F. Kosaka 1988 Endotoxin induced zinc accumulation by liver cells is mediated by metallothionein synthesis. *Biochem. Biophys. Res. Commun.*, **152**: 874–878
- Gopalakrishnan, S., H. Thilagam and P. V. Raja 2008 Comparison of heavy metal toxicity in life stages (spermiotoxicity, egg toxicity, embryotoxicity and larval toxicity) of *Hydroides elegans*. *Chemosphere*, **71**: 515–528
- Hamer, D. H. 1986 Metallothionein. *Ann. Rev. Biochem.*, **55**:

- 913–951
- Karin, M., A. Haslinger, A. Heguy, T. Dietlin and T. Cooke 1987 Metal responsive elements act as positive modulators of human metallothionein-IIA enhancer activity. *Mol. Cell. Biol.*, **7**: 606–613
- Kay, J., M. W. Brown, A. Cryer, J. F. Solbe, D. Shurben, J. S. Garvey and D. G. Thomas 1987 Metallothionein gene expression and cadmium toxicity in freshwater fish. *Experientia. Suppl.*, **52**: 627–630
- Kito, H., T. Tazawa, Y. Ose, T. Sato and T. Ishikawa 1982 Protection by metallothionein against cadmium toxicity. *Comp. Biochem. Physiol.*, **73C**: 135–139
- Klaassen, C. D., J. Liu and S. Choudhuri 1999 An intracellular protein to protect against cadmium toxicity. *Annu. Rev. Pharmacol. Toxicol.*, **39**: 267–294
- Kobayashi, N. 1971 Fertilized sea urchin eggs as indicatory materials for marine pollution bioassay, preliminary experiment. *Mar. Biol. Lab.*, **18**: 379–406
- Kobayashi, N. 1973 Studies on the effects of some agents on fertilized sea urchin eggs, as a part of the bases for marine pollution bioassay. *Mar. Biol. Lab.*, **21**: 109–114
- Kobayashi, N. 1980 Comparative sensitivity of various developmental stages of sea urchins to some chemicals. *Mar. Biol.*, **58**: 163–171
- Kobayashi, N. 1994 Application of eggs of the sea urchin, *Diadema setosum* in marine pollution bioassays. *Mar. Biol. Cent. Res. Bull.*, **59**: 91–94
- Lee, S. H. 2006 Differential gene expression in nickel (II)-treated normal rat kidney cells. *Res. Commun. Mol. Pathol. Pharmacol.*, **119**: 77–87
- Léonard, A., G. B. Gerber and P. Jacquet 1981 Carcinogenicity, mutagenicity and teratogenicity of nickel. *Mutat. Res.*, **87**: 1–15
- Lu, C. C., N. Matsumoto and S. Iijima 1979 Teratogenic effects of nickel chloride on embryonic mice and its transfer to embryonic mice. *Teratology*, **19**: 137–142
- Luo, S. Q., M. C. Plowman, S. M. Hopfer and F. W. Jr. Sunderman 1993 Mg(2+)-deprivation enhances and Mg(2+)-supplementation diminishes the embryotoxic and teratogenic effects of Ni²⁺, Co²⁺, Zn²⁺, and Cd²⁺ for frog embryos in the FETAX assay. *Ann. Clin. Lab. Sci.*, **23**: 121–129
- Okubo, K. and T. Okubo 1962 Study on the bioassay method for the evaluation of water pollution. *Bull. Tokai Reg Fish Res. Lab.*, **32**: 131–140
- Olsson, P. E., M. Zafarullah and L. Gedamu 1989 A role of metallothionein in zinc regulation after oestradiol induction of vitellogenin synthesis in rainbow trout, *Salmo gairdneri*. *Biochem. J.*, **257**: 555–559
- Pagano, G., M. Cipollaro, G. Corsale, A. Esposito, E. Ragucciand and G. G. Giordano 1985a pH-induced changes in mitotic and developmental patterns in sea urchin embryogenesis, I. Exposure of embryos. *Teratogenesis Carcinog Mutagen*, **5**: 101–112
- Pagano, G., M. Cipollaro, G. Corsale, A. Esposito, E. Ragucciand and G. G. Giordano 1985b pH-induced changes in mitotic and developmental patterns in sea urchin embryogenesis, II. Exposure of sperm. *Teratogenesis Carcinog Mutagen*, **5**: 113–121
- Phillips, B. M., P. A. Nicely, J. W. Hunt, B. S. Anderson, R. S. Tjeerdema, S. E. Palmer, F. H. Palmer and H. M. Puckett 2003 Toxicity of cadmium-copper-nickel-zinc mixtures to larval purple sea urchins. *Bull. Environ. Contam. Toxicol.*, **70**: 592–599
- Russo, R., R. Bonaventura, F. Zito, H. C. Schröder, I. Müller, W. E. G. Müller and V. Matranga 2003 Stress to cadmium monitored by metallothionein gene induction in *Paracentrotus lividus* embryos. *Cell Stress & Chaperones*, **8**: 232–241
- Saillenfait, A. M., J. P. Sabate, I. Langonne and J. de Ceaurriz 1991 Nickel chloride teratogenesis in cultured rat embryos. *Toxicol. In Vitro*, **5**: 83–89
- Saillenfait, A. M., J. P. Payan, J. P. Sabate, I. Langonne, J. P. Fabry and D. Beydon 1993 Specific amino acids modulate the embryotoxicity of nickel chloride and its transfer to the rat embryo in vitro. *Toxicol. Appl. Pharmacol.*, **123**: 299–308
- Sato, M., M. Sasaki, T. Oguro, T. Kuroiwa and T. Yoshida 1995 Induction of metallothionein synthesis by glutathione depletion after *trans*- and *cis*-stilbene oxide administration in rats. *Chem. Biol. Interact.*, **98**: 15–25
- Sunderman, F. W. Jr., S. K. Shen, M. C. Reid and P. R. Allpass 1980 Teratogenicity and embryotoxicity of nickel carbonyl in Syrian hamsters. *Teratog carcinog Mutagen*, **1**: 223–233
- Tabata, K. 1956 Systematic studies on toxic components in industrial wastes with reference to the tolerance of aquatic lives. *Bull. Tokai. Reg. Fish. Res. Lab.*, **42**: 17–21
- Takaishi, M., M. Sawada, A. Shimada, J. S. Suzuki, M. Satoh and H. Nagase 2009 Protective role of metallothionein in benzo(a)pyrene-induced DNA damage. *J. Toxicol. Sci.*, **34**: 449–458
- Zhu, Z. and D. J. Thiele 1996 Toxic metal-responsive gene transcription. *EXS.*, **77**: 307–320