*Dynamic Soil, Dynamic Plant* ©2010 Global Science Books

# Earthworms, as a Bio-monitor of Metal Contamination in Soil

**Takeshi Hirano[1*] • Kazuyoshi Tamae[2]**

[1] Department of Life and Environment Engineering, Faculty of Environmental Engineering, The University of Kitakyushu, Kitakyushu, Fukuoka, 808-0135, Japan
[2] Division of Teacher Training, Faculty of Education and Culture, University of Miyazaki, Miyazaki, 889-2192, Japan

*Corresponding author*: * **t-hirano@env.kitakyu-u.ac.jp**

## ABSTRACT

Although heavy metal pollution of soil causes biological problems, such as genotoxicity to living organisms, including human beings, few methods have been developed to assess metal mutagenicity in soil. To avoid metal mutagenicity, an adequate bio-monitoring method is required. Earthworms can be used as a bio-monitor of metal contamination in soil, because significant positive correlations have been found between the concentrations of metals, such as cadmium, copper, lead, and zinc, in the earthworm and the concentrations of these metals in the soil. Although many studies have been performed to establish bio-monitoring methods using earthworms, there have been few reports of bio-monitoring for soil mutagenicity. Recently, we examined the possibility of using earthworms in a bio-monitoring method for mutagenicity due to soil pollutants, such as metals. In the study, we analyzed the generation of 8-oxoguanine (8-oxo-Gua) in earthworms exposed to cadmium and nickel in soil. 8-Oxo-Gua is a major premutagenic form of oxidative DNA damage that induces GC-to-TA point mutations in genome DNA, leading to carcinogenesis. In this review, the use of earthworms as a bio-monitoring method for metal pollution in soil is described.

**Keywords:** bio-monitor, earthworm, metal, oxidative DNA damage, DNA repair, 8-oxoguanine
**Abbreviations: 8-oxo-Gua**, 8-oxoguanine; **ROS**, reactive oxygen species

## CONTENTS



## INTRODUCTION

Metal pollution of soil is widespread across the globe and has caused biological problems in plant nutrition and food chains, leading to potential toxicity to human beings and other living organisms. It has been reported that the atmospheric input of metals to agricultural systems also significantly contributed to pollution of soil (Vidovic 2005). These complicated pathways of contamination make it difficult to avoid exposure to metals existing in the environment. In particular, metal-induced mutagenicity is a serious problem for the diversity of living organisms and human health, because it induces gene-associated diseases, including cancer.

To avoid metal toxicity, we should first understand the features of metals and assess metal toxicity. However, the methods used for the assessment of metal toxicity are often not sensitive enough, because metals can be toxic below the technical detection limits. To overcome this limitation, many research efforts have been made to develop detection techniques or assessment methods for metal contamination of soil. Furthermore, the toxic action of metals sometimes depends on their metabolites generated in living organisms. Thus, adequate methods to assess metal toxicity are difficult to develop.

A bio-monitoring method would be appropriate to evaluate metal toxicity, because of its sensitivity and availability for unknown metabolites. Organisms such as fish, snails, and plants have been employed as bio-monitors (Harnly 1997; Citterio 2002; Regoli 2006). Although this approach is useful and promising, it is also somewhat limited, because it could be available only for a specific combination of a living organism with certain substances. Hence, it is important to find adequate living organisms as bio-monitors for each assessment.

Recent research has indicated that the earthworm is a candidate organism as a bio-monitor for soil contaminants, because it plays an important role in the soil macrofauna biomass. In addition, significant positive correlations have been found between the metal concentrations in the earthworm and the cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) concentrations in the soil (Morgan and Morgan 1988). The species *Eisenia fetida* (*E. fetida*) is most commonly used in ecotoxicology, and is recognized as a useful bio-monitor for testing the chemical toxicity of soil (Brulle *et al.* 2006). In particular, this species' proximity to the soil contaminants is a merit for the analysis (Reinecke and Reinecke 2004; Steenbergen *et al.* 2005; Hirano and Tamae 2010).

Generation of reactive oxygen species (ROS) is one of mechanisms of metal-induced toxicity. When ROS attack DNA, oxidized bases are frequently generated (Bohr *et al.*

Received: 30 April, 2010. Accepted: 23 August, 2010.

Invited Mini-Review

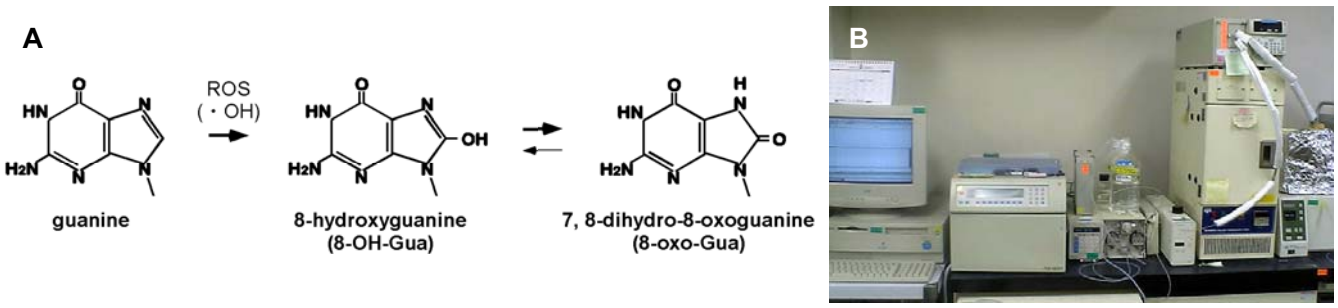


**Fig. 1** (**A**) Structure of 8-oxo-Gua. 8-Oxo-Gua is formed by the hydroxylation of guanine at the C-8 position. (**B**) The system of HPLC coupled with ECD for measurement of 8-oxo-Gua.

2002). It is believed that 8-oxo-2'-deoxyguanine (8-oxo-Gua), a major form of oxidative DNA damage, may have an important role in carcinogenesis, because it causes the GC to TA transversion type point mutation (Wood *et al.* 1990; Shibutani *et al.* 1991; Cheng *et al.* 1992). Previous work suggested that the measurement of the 8-oxo-Gua level in DNA is a useful marker for ROS stresses on DNA.

To prevent 8-oxo-Gua-induced mutations, there exist repair systems. Interestingly, some heavy metals are known to affect 8-oxo-Gua repair systems, leading to 8-oxo-Gua accumulation. Heavy metals such as arsenic (As) (Mei *et al.* 2002; Hirano 2006), cadmium (Cd) (Hirano *et al.* 1997; Mei *et al.* 2002; Potts *et al.* 2003; Youn *et al.* 2005), chromate (Cr) (Hodges and Chipman 2002; Lee *et al.* 2005), manganese (Mn) (Sava *et al.* 2004), and lead (Pb) (Sanchez-Ramos *et al.* 1994) inhibit 8-oxo-Gua excision repair activity or down-regulate the expression of 8-oxoguanine DNA glycosylase 1 (OGG1), a major repair enzyme for 8-oxo-Gua. Among these metals, the effects of Cd on 8-oxo-Gua repair systems were well documented. In our and other laboratories, it was found that Cd inhibited 8-oxo-Gua excision repair activity or 8-oxo-dGTPase activity, leading to 8-oxo-Gua accumulation in DNA (Hirano *et al.* 1997; Bialkowski *et al.* 1999; Mei *et al.* 2002; Youn *et al.* 2005). This inhibitory action of Cd on the DNA repair system might be involved in Cd carcinogenesis.

In our previous study, we analyzed 8-oxo-Gua accumulated in the DNA of *E. fetida* exposed to heavy metals, to determine if a method using earthworms as a bio-monitor is useful for the assessment of soil mutagenicity (Nakashima *et al.* 2008). We employed Cd and Ni as test metals, because the carcinogenic potentials of Cd and Ni have been established for humans and animals (**Table 1**), and these metals are known to generate 8-oxo-Gua in DNA (Hirano *et al.* 1997; Dalley and Hartwig 1997; Merzenich *et al.* 2001; Hengstler *et al.* 2003).

This review focuses on the possibility of using earthworms as a bio-monitoring method for oxidative DNA damage-inducing factors in soil.

**Table 1** IARC assessment for heavy metals.

| CAS No | Heavy metals | Group |
|---|---|---|
| 007440-38-2 | Arsenic and arsenic compounds | 1 |
| 007440-41-7 | Beryllium and beryllium compounds | 1 |
| 007440-43-9 | Cadmium and cadmium compounds | 1 |
| | Chromium [III] compounds | 3 |
| | Chromium [VI] | 1 |
| 007440-47-3 | Chromium, metallic | 3 |
| 007440-48-4 | Cobalt and cobalt compounds | 2B |
| 007439-92-1 | Lead | 2B |
| | Lead compounds, inorganic | 2A |
| | Lead compounds, organic | 3 |
| 007439-97-6 | Mercury and inorganic mercury compounds | 3 |
| | Methylmercury compounds | 2B |
| | Nickel compounds | 1 |

Data from AGENTS REVIEWED BY THE IARC MONOGRAPHS, last updated: 2 April 2009

## 8-OXOGUANINE

Point mutations generated via oxidative DNA damage are involved in cancer development, because mutations are a common feature of human cancers. In this context, the studies of 8-oxo-Gua, which is oxidized guanine, have significant implications for understanding the mechanisms of mutation-associated diseases, especially cancer (Tsuzuki *et al.* 2007). 8-Oxo-Gua is a mutagenic lesion formed spontaneously in the genomic DNA of aerobic organisms (**Fig. 1A**) and by the actions of exogenous factors, such as ionizing radiation, chemical pollutants, metals, food, and bacteria. Although 8-oxo-Gua is not necessarily the most abundant form of oxidative DNA damage, it has been the most extensively studied, because it can be quantitated with high sensitivity by high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) (**Fig. 1B**), and is quite easily measured in laboratories (Floyd *et al.* 1986; Marnett 2000). 8-Oxo-Gua and 8-oxoadenine (8-oxo-Ade) have been well studied in mutagenic oxidized DNA products, and their frequencies of generation in mammalian DNA and degrees of mutagenicity are similar (Kamiya *et al.* 1995a, 1995b; Wang *et al.* 1995; Jaruga and Dizdaroglu 1996).

Since 8-oxo-Gua was discovered, this form of DNA damage and its repair systems have been studied vigorously. 8-Oxo-Gua induces GC-to-TA transversion type point mutations (Cheng *et al.* 1992), and thus it is believed to have a key role in cancer development. Moreover, 8-oxo-Gua is efficiently removed from DNA via the short-patch base excision repair (BER) pathway, initiated by 8-oxoguanine DNA glycosylase 1 (OGG1).

## METALS AND 8-OXOGUANINE / 8-OXO-GUA REPAIR SYSTEM

We previously reported the relationship between 8-oxo-Gua/its repair ability and some metals (Hirano *et al.* 1997; Bialkowski *et al.* 1999; Mei *et al.* 2002; Youn *et al.* 2005). In the studies, we found that cadmium chloride and arsenic compounds increased the level of 8-oxo-Gua accumulation (Hirano *et al.* 1997; Mei *et al.* 2002; Hirano *et al.* 2006). It is noteworthy that these metals inhibited the 8-oxo-Gua repair activity. Some metals, such as hexavalent chromium (CrVI), manganese (Mn), and Pb, as well as Cd and arsenic (As), also reportedly inhibited the 8-oxo-Gua repair system (Hodges and Chipman 2002; Sava *et al.* 2004; Lee *et al.* 2005; Bolin *et al.* 2006; Singh *et al.* 2009). Among metals, the association of Cd with 8-oxo-Gua repair systems has been studied from the early stage of the research. In 1997, we first described an association between Cd exposure and the inhibition of 8-oxo-Gua excision repair activity in rat testes (Hirano *et al.* 1997). After the cloning of mammalian OGG1, it was demonstrated that Cd exposure down-regulated OGG1 expression in rat lung and alveolar epithelial cells (Potts *et al.* 2003). Youn *et al.* suggested that Cd attenuated the removal of γ-ray-induced 8-oxo-Gua adducts, which in turn increased the mutation frequency, and that this effect might, at least in part, result from the suppression

of hOGG1 transcription via the inactivation of the Spl transcription factor, as a result of Cd treatment (Youn *et al.* 2005). These inhibitory effects of Cd on OGG1 activity are similar to the inhibition of 8-oxo-dGTPase activity induced by Cd treatment, which led to the accumulation of 8-oxo-Gua in DNA (Bialkowski *et al.* 1999). Although it is likely that Cd exposure might broadly disturb the 8-oxo-Gua repair system, the exact mechanism of the inhibition remains unclear (Hirano 2008).

## POSSIBLE UTILITY OF USING EARTHWORMS AS A BIO-MONITOR OF METAL CONTAMINATION IN SOIL

Earthworms have been indicated as a candidate organism as a bio-monitor for soil contaminants. To establish bio-monitoring system using earthworms, the effects of contaminants on earthworms have been studied. The accumulations of both natural and depleted uranium in the earthworms were studied to evaluate corresponding biological effects (Giovanetti *et al.* 2010). The study showed that no effects were observed in terms of mortality or weight reduction, but cytotoxic and genetic effects were identified at quite low natural uranium concentrations. Among metals, methyl mercury might be more easily absorbed by and accumulated in earthworms (Zhang *et al.* 2009), suggesting that earthworm were ideal candidate for monitoring of methyl mercury. Lee *et al*. also suggested that metal bioaccumulation by earthworms can be used as ecological indicator of metal availability (Lee *et al.* 2009). In addition, it was reported that no effects of metal pollution on earthworm communities (van Gestel *et al.* 2009). These findings prompt us to recognize usefulness of earthworms as a bio-monitor. However, methods of using earthworms as a bio-monitor have not been established. Recently, effects of Cd and Pb on DNA damage generation in *E. fetida* were reported (Li *et al.* 2009). However, because they employed comet assay, the detailed form of DNA damages were unclear.

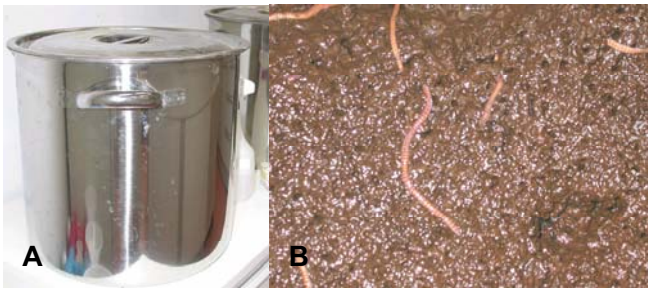


**Fig. 2** (**A**) *E. fetida* were kept in a 20 liter stainless steel tank. (**B**) *E. fetida*. The earthworm's body was cut into four rough segments: head region (S1), anterior body region (S2), posterior body region (S3), and tail region (S4). They were weighed under wet conditions and quickly frozen at – 80°C.

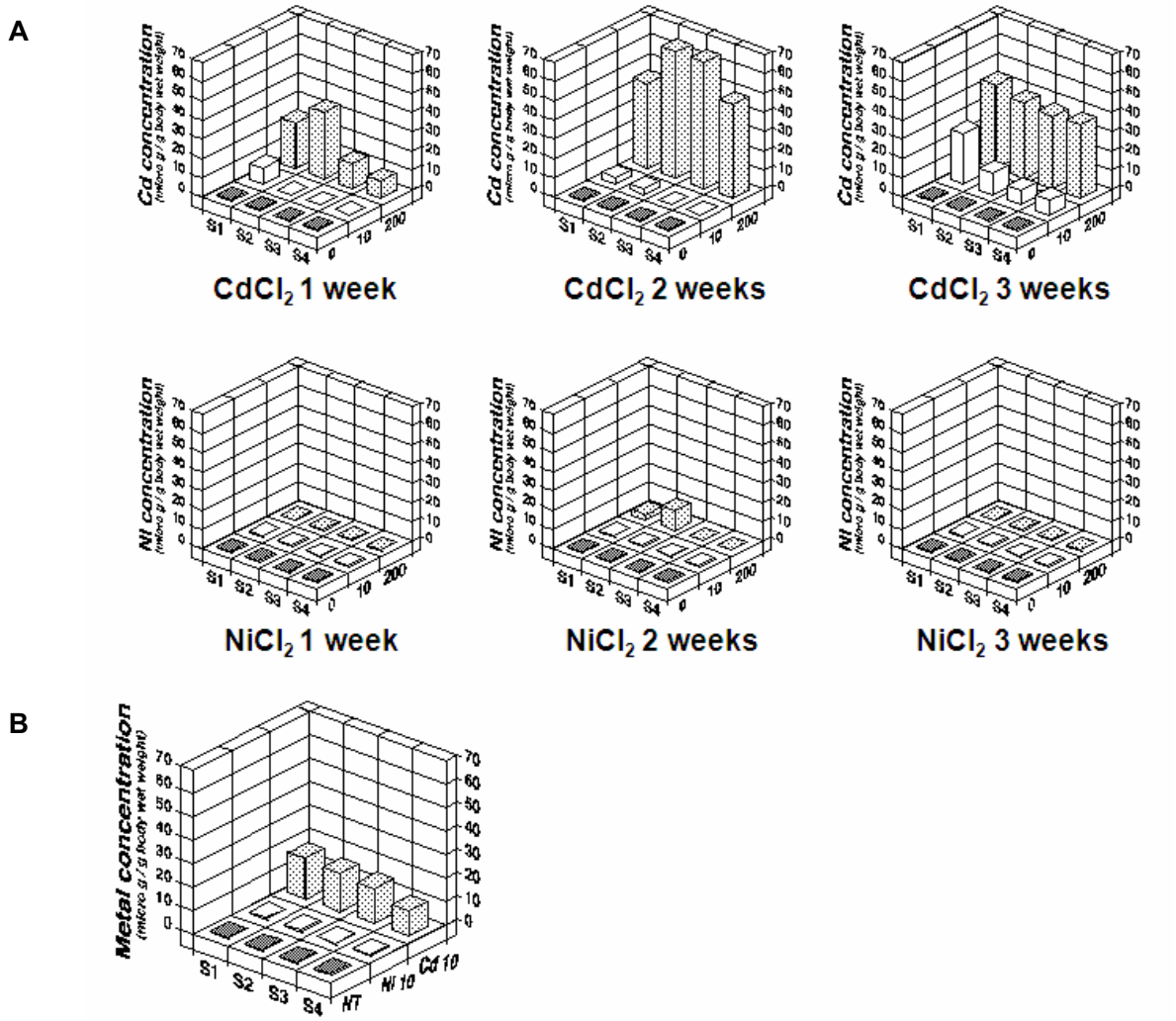


**Fig. 3** Heavy metal accumulation in *E. fetida* in the short-term experiment (**A**) and in the long-term experiment (**B**). Each data point represents the mean of three individuals. Heavy metal concentrations were measured by atomic absorption spectrometry, and are expressed as μg per body weight. Figure reproduced from **Nakashima T, Okada T, Asahi J, Yamashita A, Kawai K, Kasai H, Matsuno K, Gamou S, Hirano T** (2008) 8-Hydroxydeoxyguanosine generated in the earthworm *Eisenia fetida* grown in metal-containing soil. *Mutation Research* **654**, 138-144, ©2008, with kind permission from Elsevier Science Ltd., license number: 2492170100364.

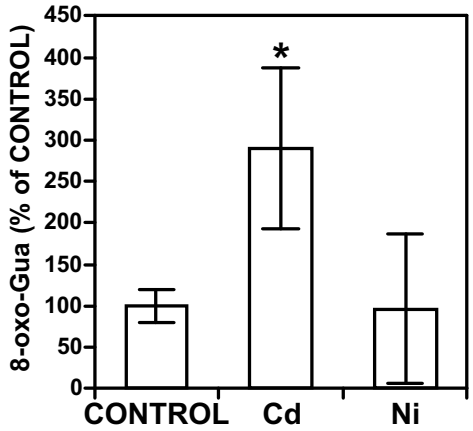


**Fig. 4** The 8-oxo-Gua levels in DNA from the S1 region of earthworms (3 month-experiment) were analyzed by HPLC equipped with electrochemical detection. The values were expressed as % of control value. Mean values ± SD, n=5. Significant differences from the control group and Ni-treated group: *$p < 0.005$ *vs*. control group, $p < 0.05$ *vs*. Ni-treated group. Figure reproduced from **Nakashima T, Okada T, Asahi J, Yamashita A, Kawai K, Kasai H, Matsuno K, Gamou S, Hirano T** (2008) 8-Hydroxydeoxyguanosine generated in the earthworm *Eisenia fetida* grown in metal-containing soil. *Mutation Research* **654**, 138-144, ©2008, with kind permission from Elsevier Science Ltd., license number: 2492170100364.

## 8-OXOGUANINE GENERATED IN EARTHWORMS

Among the many kinds of organisms living in soil, the earthworm is a most useful organism for the evaluation of metal contamination, because significant positive correlations have been found between the metal concentrations in the earthworm and the soil Cd, Cu, Pb and Zn concentrations (Morgan and Morgan 1988).

We recently analyzed the 8-oxo-Gua accumulated in the DNA of *E. fetida* exposed to metals, to determine if a method using earthworms as a bio-monitor is useful for the assessment of soil mutagenicity (Nakashima *et al.* 2008). In the study, *E. fetida* were kept in a 20 liter stainless steel tank at an ambient temperature of 24°C, using mold with skim milk as a food source until metal exposure (**Fig. 2**). Three to six individuals were kept in a 600 mL glass container containing 50 g of soil with/without metal. They were exposed to 10 or 200 μg metal/g soil for 1, 2, and 3 weeks or 10 μg metal/g soil for 3 months. As a result, we detected a high level of Cd accumulation in *E. fetida* (**Figs. 3A, 3B**). On the other hand, no Ni accumulation was observed (**Fig. 3**).

The 8-oxo-Gua levels in the DNA of *E. fetida* treated with Cd for 3 months were significantly higher than those in control *E. fetida* or in *E. fetida* treated with Ni (**Fig. 4**). In addition, we observed positive staining of 8-oxo-Gua in the seminal vesicles in almost all samples (**Fig. 5**). The positive signals in the seminal vesicles were clearly detected only in *E. fetida* treated with 10 μg of Cd at 3 months (**Fig. 5F**). The seminal vesicles are considered as metallothionein (MT)-poor organs. Therefore, it seems reasonable to speculate that a lower level of MT expression is involved in Cd-induced DNA damage accumulation.

## CONCLUSIONS

We demonstrated the possible utility of using earthworms as bio-monitors, by measuring the oxidative DNA damage generated in the earthworms, as a bio-monitoring method for assessing soil mutagenicity. However, many points remain unresolved. For example, this method could be reliable only for bio-accumulated metals, such as Cd, but not for non-bio-accumulated metals, such as Ni, even if they generate 8-oxo-Gua. To establish a broader bio-monitoring method using earthworms to assess soil mutagenicity, further studies will be required.

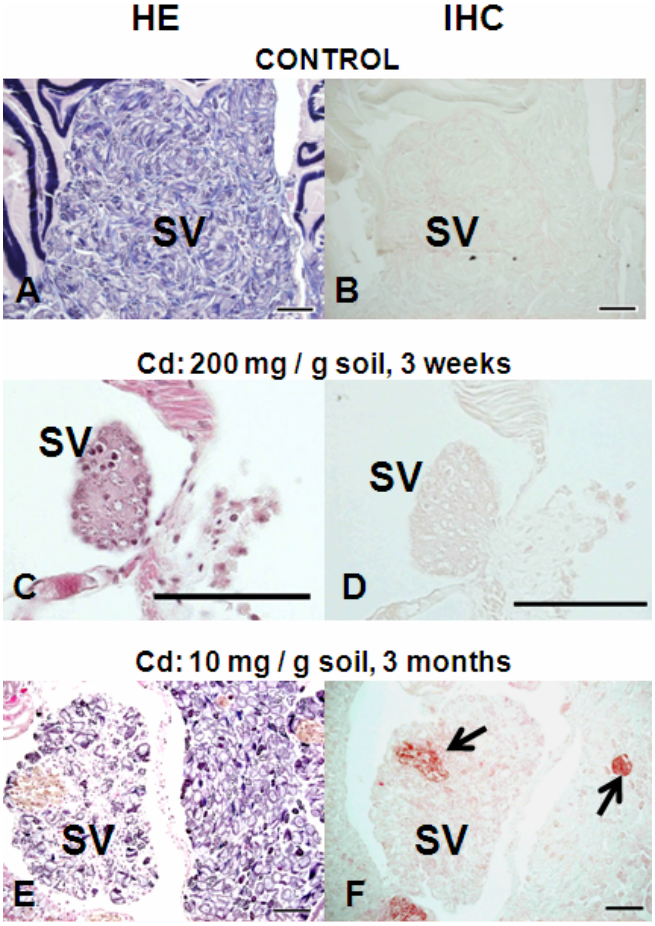


**Fig. 5** Immunohistochemical analyses of 8-oxo-Gua accumulation in the seminal vesicles of *E. fetida* (S1) treated with Cd (200 μg / g soil, 3 weeks; HE (C), IHC (D)), and Cd (10 μg / g soil, 3 months; HE (E), IHC (F)). Controls: (A) and (B). Positive signals for 8-oxo-Gua accumulation in the seminal vesicles were detected only in *E. fetida* treated with Cd (10 μg / g soil) for 3 months. HE stained section and IHC stained section are in several serial sections. Arrowhead (black) shows positive signals in seminal vesicles. SV: seminal vesicles. All scale bars are 100 μm. Figure reproduced from **Nakashima T, Okada T, Asahi J, Yamashita A, Kawai K, Kasai H, Matsuno K, Gamou S, Hirano T** (2008) 8-Hydroxydeoxyguanosine generated in the earthworm *Eisenia fetida* grown in metal-containing soil. *Mutation Research* **654**, 138-144, ©2008, with kind permission from Elsevier Science Ltd., license number: 2492170100364.

## ACKNOWLEDGEMENTS

This work was supported by a grant from The Foundation for Earth Environment. The authors thank Elsevier Ltd. to reproduce the material in Nakashima *et al.* (2008).

## REFERENCES

**Bialkowski K, Bialkowska A, Kasprzak KS** (1999) Cadmium (II), unlike nickel (II), inhibits 8-OH-dGTPase activity and increases 8-OH-dG level in DNA of the rat testis, a target organ for cadmium (II) carcinogenesis. *Carcinogenesis* **20**, 1621-1624

**Bolin CM, Basha R, Cox D, Zawia NH, Maloney B, Lahiri DK, Cardozo-Pelaez F** (2006) Exposure to lead (Pb) and the developmental origin of oxidative DNA damage in the aging brain. *FASEB Journal* **20**, 788-790

**Borh VA, Stevnsner T, de Souza-Pinto NC** (2002) Mitochondrial DNA repair of oxidative damage in mammalian cells. *Gene* **286**, 127-134

**Brulle F, Mitra G, Coquerelle C, Vieau D, Lemiere S, Leprêtre A, Vandenbulcke F** (2006) Cloning and real-time PCR testing of 14 potential biomarkers in *Eisenia fetida* following cadmium exposure. *Environmental Science and Technology* **40**, 2844-2850

**Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA** (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. *Journal of Biological Chemistry* **267**, 166-172

**Citterio S, Aina R, Labra M, Chiani A, Fumagalli P, Sgorbati S, Santagostino A** (2002) Soil genotoxicity assessment: a new strategy based on biomolecular tools and plant bioindicators. *Environmental Science and Technol-*

*ogy* **36**, 2748-2753

**Dalley H, Hartwig A** (1997) Induction and repair inhibition of oxidative DNA damage by nickel (II) and cadmium (II) in mammalian cells. *Carcinogenesis* **18**, 1021-1026

**Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC** (1986) Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. F*ree Radical Research Communications* **1**, 163-172

**Giovanetti A, Fesenko S, Cozzella ML, Asencio LD, Sansone U** (2010) Bioaccumulation and biological effects in the earthworm *Eisenia fetida* exposed to natural and depleted uranium. *Journal of Environmental Radioactivity* **101**, 509-516

**Harnly M, Seidel S, Rojas P, Fornes R, Flessel P, Smith D, Kreutzer R, Goldman L** (1997) Biological monitoring for mercury within a community with soil and fish contamination. *Environmental Health Perspectives* **105**, 424-429

**Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Götte W, Jung D, Mayer-Popken O, Fuchs J, Gebhard S, Bienfait HG, Schlink K, Dietrich C, Faust D, Epe B, Oesch F** (2003) Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. *Carcinogenesis* **24**, 63-73

**Hirano T, Yamaguchi Y, Kasai H** (1997) Inhibition of 8-hydroxyguanine repair in testes after administration of cadmium chloride to GSH-depleted rats. *Toxicology and Applied Pharmacology* **147**, 9-14

**Hirano T, Kawai K, Ootsuyama Y, Kasai H** (2006) Fragmentation of the DNA repair enzyme, OGG1, in mouse nonparenchymal liver cells by arsenic compounds. *Genes and Environment* **28**, 62-67

**Hirano T** (2008) Repair system of 7, 8-dihydro-8-oxoguanine as a defense line against carcinogenesis. *Journal of Radiation Research* **49**, 329-340

**Hirano T, Tamae K** (2010) Heavy metal-induced oxidative DNA damage in earthworms: A review. *Applied and Environmental Soil Science* **2010**, Article ID 726946

**Hodges NJ, Chipman JK** (2002) Down-regulation of the DNA –repair endonuclease 8-oxoguanine DNA glycosylase 1 (hOGG1) by sodium dichromate in cultured human A549 lung carcinoma cells. *Carcinogenesis* **23**, 55-60

**Jaruga P, Dizdaroglu M** (1996) Repair of products of oxidative DNA base damage in human cells. *Nucleic Acids Research* **24**, 1389-1394

**Kamiya H, Murata-Kamiya N, Koizume S, Inoue H, Nishimura S, Ohtsuka E** (1995a) 8-Hydroxyguanine (7, 8-dihydro-8-oxoguanine) in hot spots of the c-Ha-ras gene: effects of sequence contexts on mutation spectra. *Carcinogenesis* **16**, 883-889

**Kamiya H, Miura H, Murata-Kamiya N, Ishikawa H, Sakaguchi T, Inoue H, Sasaki T, Masutani C, Hanaoka F, Nishimura S, Ohtsuka E** (1995b) 8-Hydroxyadenine (7, 8-dihydro-8-oxoadenine) induces misincorporation in *in vitro* DNA synthesis and mutations in NIH 3T3 cells. *Nucleic Acids Research* **23**, 2839-2899

**Lee AJ, Hodges NJ, Chipman JK** (2005) Interindividual variability in response to sodium dichromate-induced oxidative DNA damage: Role of the $Ser^{326}Cys$ polymorphism in the DNA-repair protein of 8-oxo-7, 8-dihydro-2'-deoxyguanosine DNA glycosylase 1. *Cancer Epidemiology, Biomarkers and Prevention* **14**, 497-505

**Lee SH, Kim EY, Hyun S, Kim JG** (2009) Metal availability in heavy metal-contaminated open burning and open detonation soil: assessment using soil enzymes, earthworms, and chemical extractions. *Journal of Hazardous Materials* **170**, 382-388

**Li M, Liu Z, Xu Y, Cui Y, Li D, Kong Z** (2009) Comparative effects of Cd and Pb on biochemical response and DNA damage in the earthworm *Eisenia fetida* (Amelida, Oligocheta). *Chemosphere* **74**, 621-625

**Marnett LJ** (2000) Oxyradicals and DNA damage. *Carcinogenesis* **21**, 361-370

**Mei N, Kunugita N, Hirano T, Kasai H** (2002) Acute arsenite-induced 8-hydroxyguanine is associated with inhibition of repair activity in cultured human cells. *Biochemical and Biophysical Research Communications* **297**, 924-930

**Merzenich H, Hartwig A, Ahrens W, Beyersmann D, Schlepegrell R, Scholze M, Timm J, Jöckel KH** (2001) Biomonitoring on carcinogenic metals and oxidative DNA damage in a cross-sectional study. *Cancer Epidemiology, Biomarkers and Prevention* **10**, 515-522

**Morgan JE, Morgan AJ** (1988) Earthworms as biological monitors of cadmium, copper, lead and zinc in metalliferous soils. *Environmental Pollution* **54**, 123-138

**Nakashima T, Okada T, Asahi J, Yamashita A, Kawai K, Kasai H, Matsuno K, Gamou S, Hirano T** (2008) 8-Hydroxydeoxyguanosine generated in the earthworm *Eisenia fetida* grown in metal-containing soil. *Mutation Research* **654**, 138-144

**Potts RJ, Watkin RD, Hart BA** (2003) Cadmium exposure down-regulates 8-oxoguanine DNA glycosylase expression in rat lung and alveolar epithelial cells. *Toxicology* **184**, 189-202

**Regoli F, Gorbi S, Fattorini D, Tedesco S, Notti A, Machella N, Bocchetri R, Benedetti M, Piva F** (2006) Use of the land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicologic effects of urban pollution: an integrated approach. *Environmental Health Perspectives* **114**, 63-69

**Reinecke AJ, Reinecke SA** (2004) Earthworm as test organisms in ecotoxicological assessment of toxicant impacts on ecosystems. In: Edwards CA (Ed) *Earthworm Ecology*, CRC Press LLC, Boca Baton, FL, pp 299-320

**Sanchez-Ramos J, Overvik E, Ames BN** (1994) A marker of oxyradical-mediated DNA damage (oxo8dG) is increased in nigrostratum of Parkinson's disease brain. *Neurodegeneration (Experimental Neurology)* **3**, 197-204

**Sava V, Mosqouera D, Song S, Cardozo-Pelaez F, Sánchez-Ramos JR** (2004) Effects of melanin and manganese on DNA damage and repair in PC12-derived neurons. *Free Radical Biology and Medicine* **36**, 1144-1154

**Shibutani S, Takeshita M, Grollman AP** (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-OH-dG. *Nature* **349**, 431-434

**Singh KP, Kumari R, Pevey C, Jackson D, du Mond JW** (2009) Long duration exposure to cadmium leads to increased cell survival, decreased DNA repair capacity, and genomic instability in mouse testicular Leydig cells. *Cancer Letters* **279**, 84-92

**Steenbergen NTTM, Iaccino F, De Winkel M, Reijnders L, Peijnenburg WJGM** (2005) Development of a biotic ligand model and a regression model predicting acute copper toxicity to the earthworm *Aporrectodea caliginosa*. *Environmental Science and Technology* **39**, 5694-5702

**Tsuzuki T, Nakatsu Y, Nakabeppu Y** (2007) Significance of error-avoiding mechanisms for oxidative DNA damage in carcinogenesis. *Cancer Sciences* **98**, 465-470

**Van Gestel CA, Koolhaas JE, Hamers T, van Hoppe M, van Roovert M, Korsman C, Reinecke SA** (2009) Effects of metal pollution on earthworm communities in a contaminated floodplain area: linking biomarker, community and functional responses. *Environmental Pollution* **157**, 895-903

**Vidovic M, Sadibasic A, Cupic S, Lausevic M** (2005) Cd and Zn in atmospheric deposit, soil, wheat, and milk. *Environmental Research* **97**, 26-31

**Wang YJ, Ho YS, Lo MJ, Lin JK** (1995) Oxidative modification of DNA bases in rat liver and lung during chemical carcinogenesis and aging. *Chemico-Biological Interactions* **94**, 135-145

**Wood ML, Dizdarogle M, Gajewski E, Essigmann JM** (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry* **29**, 7024-7032

**Youn CK, Kim SH, Lee DY, Song SH, Chang IY, Hyun JW, Chung MH, You HJ** (2005) Cadmium down-regulates human OGG1 through suppression of Sp1 activity. *Journal of Biological Chemistry* **280**, 25185-25195

**Zhang ZS, Zheng DM, Wang QC, Lv XG** (2009) Bioaccumulation of total and methyl mercury in three earthworm species (*Drawida* sp., *Allolobophora* sp., and *Limnodrilus* sp.). *Bulletin of Environmental Contamination and Toxicology* **83**, 937-942