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Possible Implications of Nitrate Contaminated Water to Human Health- A Review

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Abstract

Water is one of the fundamental resources for maintaining the life on earth and therefore preserving the availability and quality of this resource is extremely important. Due to anthropogenic activities like intensive use of agrochemicals mainly nitrogenous fertilizers, discharge of municipal and domestic sewage, septic systems, road and buildings processes, it could be chemically, physically, or microbiologically contaminated. Each of which is linked to various sources and health related problems and consequences. Long-term exposure to high nitrate drinking water may increase human health risks which may lead to chronic poisoning, linked to methaemoglobinemia. Nitrate contamination in groundwater and surface water is extensively explored problem at global level. Water containing high concentration of nitrate is unfit for human consumption and can contribute to eutrophication if discharging to fresh water sources. The objective of this review paper is to study the possible implications of nitrate present in different water sources to human health.

Key words: Nitrate contamination, water sources, methaemoglobinemia, human health

1. Introduction

Out of the fundamental resources like air, soil and water, water is an important resource as sustainable social and economic growth of mankind is largely dependent on it. However, securing water quality to satisfy the needs of humans and ecosystems is one of the primary challenging issues of the 21st century **(Amangabara and Ejenma, 2012).** About 70% of Earth's surface is covered by water of which 97.5% is salty and 2.5% is fresh water and Less than 1% of this 2.5% amount of freshwater is accessible **(Mishra and Dubey 2015).** Ground water accounts for about 98% of global freshwater on Earth **(Lui *et al.* 2011)** which is used by approximately 2.5 billion people worldwide for their daily needs **(Margat, 2008, Margat and van der Gun, 2013).** Assessment of water quality is necessity in order to provide quality drinking water intended for human consumption and for other usual domestic purposes **(Jain *et al*. 2009).**

Consumption of contaminated deterioting water quality affects livehoods at risk **(Leelavathi *et al.* 2016).** Nitrate contamination is one of the most widespread groundwater problems worldwide **(Almasri, 2007; Gupta *et al.*, 2008).** Water containing high concentration of nitrate is unfit for human consumption and can contribute to eutrophication if discharging to fresh water sources due to excess nutrients **(Vitousek *et al.*, 1997; WHO, 1999; Mason, 2002).** Primary factors responsible for nitrate in groundwater include fertilizers, septic systems, and manure storage or spreading operations. Inverse relationships between nitrate concentration and aquifer depth have been reported in previous studies **(Spalding and Exner 1993; Ray and Schock 1996; Ako *et al.* 2011).** The contaminants coming from these various sources can be transported to surface waters by surface runoff and groundwater discharge. Several studies indicate that rural land uses, especially agricultural practices, can cause nitrate contamination of underlying groundwater. In addition, regions experiencing significant population growth may be substantially contributing to the groundwater nitrate contamination **(Ako *et al*. 2011).**

The World Health Organization has set the limit values for nitrate and nitrite in drinking water to 50 and 3 mg/l, respectively. These values are based on epidemiological evidence for methemoglobinemia

(WHO, 2007). Nitrate levels at or above this level have been known to cause a potentially fatal health disorder in infants under six months of age called Methaemoglobinemia or "blue-baby" syndrome (reduction in the oxygen carrying capacity of blood) **(Fan and Steinberg, 1996; WHO, 1999; WHO, 2004; Horing and Chapman, 2004).** Therefore, the nitrate pollution is a severe environmental problem that should be of high concern.

2. Source of contamination

Nitrate contamination is a common water problem throughout the world **(Burden, 1982; Spalding and Exner, 1993; Beeson and Cook, 2004)**. During the past two centuries, the man made activities has substantially altered the global nitrogen cycle thereby increasing both the availability and the mobility of nitrogen **(Vitousek *et al*., 1997; Carpenter *et al*., 1998; Galloway and Cowling, 2002).** Nitrate (NO_3^-) is very soluble in water and is negavely charged and thus has a high mobility and potential for loss from the unsaturated zone by leaching and can remains in groundwater for decades **(Manassaram *et al.*, 2006).** Nitrate in ground water has a variety of sources classified as point source (from a single, identifiable source) and non-point source (from many diffuse sources). Point source includes livestock farms, sewage disposal systems, including septic tanks, slurry lagoons and non-point source includes fertilized cropland, land runoff or naturally occurring sources of nitrogen such as precipitation, atmospheric deposition, drainage, seepage, or hydrological modification **(Manassaram *et al*., 2006; Yang *et al*., 2007; Gupta *et al*., 2008).** Alternative sources such as sewer breakthrough and animal waste can also cause a significant increase of nitrate concentrations **(Hudak and Blanchard 1997).**

Contamination of nitrate was also determined by the depths of well and borehole **(Kross *et al.* (1993)**. Such a distribution clearly indicates that nitrate species in most of the considered groundwaters are of superficial origin (anthropogenic).

High concentration of nitrate can result in many environmental and ecological problems, such as blooms of toxic algae, eutrophication of lakes and reservoirs and extinction of species in the river ecosystem **(Gautam and Iqbal, 2010, Li *et al.*, 2013).** In addition, long-term exposure to high nitrate drinking water may increase human health risks **(Carpenter *et al*., 1998, Li *et al*., 2014),** which may lead to chronic poisoning, linked to Methaemoglobinemia **(Itoh *et al*., 2011, Yang *et al*., 2012, Liu *et al*., 2013).** To prevent these deleterious effects of nitrate on human health, drinking water quality criteria have been established: the USA federal maximum contaminant level is 10 mg NO3-N/l **(US Environmental Protection Agency, 1986; Nash, 1993; Scott and Crunkilton, 2000).**

3. Nitrogen cycle

The nitrogen cycle is one of the most important nutrient cycles in terrestrial ecosystems. Nitrates and nitrites are chemical compounds that are produced naturally in the nitrogen cycle. Nitrogen cycling involves four major steps: nitrogen fixation, ammonification, nitrification and denitrification. Use of large amounts of nitrogenous fertilizers to agricultural fields influences these processes, especially nitrification and denitrification, and results in increased production of N_2O **(Akiyama *et al*., 2006)** and pollution of ground water by leaching of nitrate from agricultural fields. In the nitrogen cycle, nitrogen from different sources such as atmosphere, animal and human wastes, decaying vegetation, and nitrate containing fertilizers are initially converted to ammonia by saprophytic soil-dwelling bacterial action in a process known as ammonification. Ammonia is subsequently converted to nitrite by soil-dwelling, nitrogen-fixing bacteria in a process known as nitrification. Nitrites may be further oxidized by soil-dwelling nitrifying bacteria and enter groundwater as nitrates. Denitrification is central to the nitrogen cycle and involves the reduction of nitrate via a chain of microbial reduction reactions to nitrogen gas **(Knowles, 1982).** The organisms capable of denitrification tend to be ubiquitous in surface water, soil and groundwater **(Beauchamp *et al*., 1989).**

The reaction mechanism for different processes of nitrogen cycle is as:

The utilization of molecular nitrogen (N_2) by particular bacteria such as *rhizobium*, cyanobacteria is called nitrogen fixation.

Reduction of $N_2 \rightarrow NH_3$ (ammonia)

Ammonia produced by this process is further converted to proteins, nucleic acids (DNA), and other nitrogen-containing organic molecules

$NH_3 \rightarrow$ nitrogenous organic molecules: proteins, nucleic acids and so forth

Nitrification is two step microbial processes in which NH_4^+ is oxidized to NO_3^-. Nitrosifyers, such as the bacterium *Nitrosomonas*, convert ammonium ion into nitrite (NO_2^-) first as:

$$2\ NH_4^+ + 3O_2 \rightarrow 2\ NO_2^- + 2H_2O + 4H^+ + \text{energy}$$

Later, nitrifying bacteria, such as the bacterium *Nitrobacter*, oxidize nitrite into nitrate as:

$2NO_2^- + O_2 \rightarrow 2\ NO_3^-$

And finally conversion of nitrate (NO_3^-) into gaseous nitrogen compounds such as N_2O, NO, and N_2 by different bacteria in soils is called denitrification, or nitrate reduction. The nitrate reduction reaction can be written as a half equation that illustrates the role of electron (e^-) transfer in the process (non-specific to the electron donor) **(Tesoriero *et al*., 2000):**

$2NO_3^- + 12H^+ + 10e^- \rightarrow N2 + 6H_2O$

4. Nitrate and health issues

The exposure to nitrate by humans is dependent on an individual's intake of vegetables and the local concentration of nitrate in drinking water as well as the total amount of NO produced in the body. Nitrate ingested from the diet is rapidly absorbed in the small intestine, mixes with the endogenous nitrate from oxidation of NO and is readily distributed throughout the body **(Walker, 1996).** About 25% of an oral nitrate load is concentrated and excreted by salivary glands **(Spiegelhalder *et al*., 1976),** so that salivary nitrate concentration is approximately 10 times higher than plasma nitrate. Approximately 20% of salivary nitrate is reduced to nitrite in the mouth by facultative anaerobic bacteria which are found on the surface of the tongue **(Bjorne *et al*., 2004; Doel *et al*., 2005; Lundberg *et al*., 2004)** resulting in about a 5% reduction of total ingested nitrate to nitrite. As a result, nitrate ingestion is the main source of nitrite exposure.

4.1. Methaemoglobinemia

Nitrate itself is not toxic to humans. It becomes a problem only when it is converted to nitrite in the human body, resulting in Methaemoglobinemia or "Blue Baby Syndrome" which cannot bind oxygen **(NAS, 1977, Majumdar, 2003, Manassaram *et al.*, 2006).**

$NO_2^- + oxyHb(Fe^{2+}) \rightarrow metHb(Fe^{3+}) + NO_3^-$

Due to the formation of methaemoglobin the oxygen delivery to tissue is impaired **(Knobelock *et al*., 2000; Speijers & Van den Brandt, 2003).** Blue baby syndrome or Methaemoglobinemia occurs if this oxidation process overwhelms the protective reduction capacity of the cells **(Jaffe, 1981, safe drinking water act of 1974).** Nitrate is converted to nitrite (under anaerobic conditions in the gut), and nitrite acts as the oxidizing agent to form methaemoglobin in the red blood cells **(Manassaram *et al.*, 2006).** Thus, nitrate reduces the total oxygen carrying capacity of the blood **(Sandstedt, 1990; Amdur *et al*., 1991; Wolfe and Patz, 2002).** As different parts of the body get deprived of oxygen, clinical symptoms of oxygen starvation start to appear, the main being cyanosis which appear when concentrations of methaemoglobin (metHb) are 15–20% of the total haemoglobin. The clinical symptoms of hypoxia, such as fatigue and dyspnoea occur at concentrations greater than 30% of the total haemoglobin. The lips or even the skin start to become blue hence the common name, the blue baby syndrome. Methaemoglobin concentrations greater than 50% can quickly lead to coma and death **(Knobelock *et al*., 2000).** In addition to drinking water, which contributes an estimated < 3–21% of the average adult intake of nitrate, other sources include vegetables, meat, and meat products preserved with sodium nitrite **(Jaffe, 1981, Wogan, 1995)**. Nitrite is also a substrate in the formation of N-nitroso compounds from nitrosatable drugs **(Brender *et al*., 2004).**

4.2. Reproductive and developmental toxicity

Several epidemiologic studies have attempted to evaluate the reproductive and developmental effects of nitrate. Nitrate, nitrite, and N-nitroso compounds may traverse the placenta and affect the fetus in utero **(Fan *et al*., 1987, Bruning-Fann and Kaneene, 1993)**. Earlier studies have reported that at extremely high levels of exposure, nitrate (NO_3^-) and nitrite (NO_2^-) produced adverse reproductive effects on test animals like rats and mice **(Fan *et al*., 1987).** Gestational studies with nitrate (NO_3^-) and nitrite (NO_2^-) showed no significant differences in fertility, birth weight, litter size and sex ratio in these test animals. However, exposure of rats to nitrite (NO_2^-) in drinking water during gestation appeared to affect pup growth and hematological status **(Roth *et al*., 1987, Roth and Smith, 1988)** while no maternal toxicity was observed. **Pant and Srivastava (2002)** have reported effects of nitrate exposure on semen quality in male mice at 900 ppm. On aquatic animals the main toxic action of nitrate is due to the conversion of oxygen-carrying pigments (e.g., haemoglobin, hemocyanin) to forms that are incapable of carrying oxygen (e.g., methemoglobin) **(Grabda *et al*., 1974; Conrad, 1990; Jensen, 1996; Scott and Crunkilton, 2000; Cheng and Chen, 2002).** Results from animal studies of both nitrite and nitrate exposure via drinking water have shown effects on male rat testosterone production and testicular size and for nitrate these effects have been seen at concentrations close to the allowed limit in drinking water **(Panesar and Chan, 2000).** Earlier studies have reported increase toxic effect to tadpoles with increasing concentrations and exposure times of nitrate **(Baker and Waights, 1994; Hecnar, 1995; Xu and Oldham, 1997; Marco *et al.*, 1999; Schuytema and Nebeker, 1999a).** The tolerance of amphibians to nitrogen fertilizers may however increase with increasing body size **(Schuytema and Nebeker, 1999b)** and environmental adaptation **(Johansson *et al.*, 2001).** Nitrogen fertilizers, such as ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$), may be contributing (with pesticides) to the decline of amphibian populations in agricultural areas **(Wederkinch, 1988; Berger, 1989; Hecnar, 1995; Oldham *et al.*, 1997; Birge *et al.*, 2000).**

Hansen *et al*., 2009 examined how *in utero* exposure to nitrate would affect male rat fetuses. In addition, endocrine disrupting activity of nitrate and nitrite were studied in two in vitro assays, the H295R assay and T-screen and found no consistent indications that nitrate induces anti-androgenic effects in male fetuses or that prenatal nitrate exposure affected the thyroid axis. The indications for endocrine disrupting effects of nitrate and nitrite and especially their ability to interfere with androgen production in males raise a concern for effects during male sexual development. The prenatal period has been shown to be the most sensitive period for effects of numerous endocrine disrupting chemicals, including certain anti-androgenic phthalates. These phthalates have been shown to decrease the anogenital distance (AGD) and impair steroidogenesis in fetal and neonatal male rats at critical periods of sexual development leading to malformations in the male reproductive system and reduced sperm count later in life **(Barlow *et al*., 2004; Borch *et al*., 2006; Carruthers and Foster, 2005; Ema *et al*., 2000, 2003; Gray *et al*., 2000; Parks *et al*., 2000).** Other adverse reproductive effects, such as mummified fetuses; lesions on the cervix, uterus, and placenta; and maternal death were also reported by earlier experimental studies reviewed by **Fan *et al.* (1987)**.

4.3. Carcinogenic effect

Nitrate has been implicated in bladder, ovarian, stomach and liver cancers **(Tsezou *et al*., 1996; Mueller *et al*., 2001; Weyer *et al*., 2001).** According to the findings of **Gulis *et al*. (2002)** on cancer, there was a positive association between nitrate level and incidence of non-hodgkin lymphoma and colon cancer, with an excess risk among women. In addition, ingested nitrates have a potential role in developing cancers of the digestive tract through their contribution to the formation of nitrosamines, which are among the most potent of the known carcinogens in mammals **(Harte *et al*., 1991; Nash, 1993).** In contrast, many epidemiological researches indicated that nitrate intake did not link to cancer. They had, however, produced contradictory effects **(Archer 2002).** Long term consumption of water at the concentration of 5.5 mg/l also would not increase the incidence of bladder cancer **(Ward *et al.* 2003).**

4.4. Thyroid

Ingestion of nitrate at high doses can competitively inhibit iodine uptake and induce hypertrophy of the thyroid gland **(De Groef *et al.* 2006).** Some epidemiological studies in children showed that the goitre develops easily in areas where the contamination of drinking water by nitrates exceeds the permissible daily limit of 50 mg/l fixed by the WHO (1998) **(Gatseva *et al*., 1998; Vladeva *et al*., 2000).** However, dietary nitrate, the predominant source of intake, was associated with increased prevalence of hypothyroidism but not hyperthyroidism. Effects on thyroid hormones and increased weight of the thyroid gland have been observed in adult female rats after nitrate exposure of 50 mg/l **(Esciocak *et al*., 2005). Zaki *et al.* (2004)** exposed male rats to nitrate in the drinking water and reported a dose-dependent increase in weight of the thyroid gland as well as decreased levels of triiodothyronine (T3) at nitrate concentrations of 150 and 500 mg/l and a decrease in thyroxine (T4) at 500 mg/l. Furthermore, increased thyroid volume has been reported in humans living in areas with high concentrations of nitrate in their drinking water. However, other possible thyroid disruptors than nitrate were not measured in the drinking water in these studies **(Tajta kova *et al*., 2006; van Maanen *et al*., 1994).**

Other health effects associated with high concentration of nitrate in drinking water as per earlier studies are type 1 childhood diabetes (T1D), blood pressure, and acute respiratory tract infections in children **(Ward *et al.* 2005).**

5. Conclusion

Ground water and surface water contamination by nitrate is a widespread problem in our world. Agricultural drainage, leachate from waste pile, and pollution from human and animal waste, are responsible for a vast increase of nitrate concentrations in different water source. Populations with the highest exposure to nitrate from their drinking water are those living in agricultural regions. By affecting the drinking water quality it can cause public health problems, and environmental degradation of ecosystems. Nitrate concentrations in global water supplies are likely to increase in the future due to population growth, increases in nitrogen fertilizer use, and increasing intensity and concentration of animal agriculture. Adverse effects of nitrate contaminated water can be minimized by proper water management and good governance to mitigate the risks for nitrate contamination. However, in order to better manage nitrate contamination in groundwater systems, researchers, water resources specialists and policy makers do need information on the scope, distribution and severity of groundwater nitrate contamination. In other words, it would be better to facilitate early diagnosis of possible changes and widen their inspiration for selecting effective measures for interventions.

References

[1] Akiyama, H., Yan, X. and Yagi, K. (2006). Estimations of emission factors for fertilizer-induced direct N_2O emissions from agricultural soils in Japan: Summary of available data. *Soil Science and Plant Nutrition* 52, 774–787.

[2] Ako, A.A., Jun, S., Takahiro, H., Kimpei, I., Nkeng, G.E., Fantong, W.Y., Eyong, G.E.T. and Ntankouo, N.R. (2011). Evaluation of groundwater quality and its suitability for drinking, domestic, and agricultural uses in the Banana Plain (Mbanga, Njombe, Penja) of the Cameroon Volcanic. *Environ Geochem Health* 33:559–575.

[3] Almasri, M.N. (2007). Nitrate contamination of groundwater: A conceptual management framework. *Environ. Impact Assess. Rev*. 27: 220-242.

[4] Amangabara, G.T. and Ejenma, E. (2012). Groundwater quality assessment of Yenagoa and environs Bayelsa State, Nigeria between 2010 and 2011. *Resour. Environ*. 2 (2), 20–29.

[5] Amdur, M.O., Dull, J., Klassen, E.D. (Eds.), (1991). Casarett and Doull's Toxicology, 4th ed. Pergamon Press, New York.

[6] Archer, D.L. (2002). Evidence that ingested nitrate and nitrite are beneficial to health. *J. Food Prot.* 65 (5), 872–875.

[7] Baker, J. and Waights, V. (1994). The effects of nitrate on tadpoles of the tree frog (Litoria caerulea). *Herpetol*. J. 4, 106–107.

[8] Barlow, N. J., McIntyre, B. S., and Foster, P. M. (2004). Male reproductive tract lesions at 6, 12, and 18 months of age following in utero exposure to di(n-butyl) phthalate. *Toxicol. Pathol*. 32, 79–90.

[9] Bjorne, H.H., Petersson, J., et al., (2004). Nitrite in saliva increases gastric mucosal blood flow and mucus thickness. *J. Clin. Invest*. 113 (1), 106–114.

[10] Borch, J., Axelstad, M., Vinggaard, A. M., and Dalgaard, M. (2006). Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis. *Toxicol. Lett.* 163, 183–90.

[11] Beauchamp, E.G., Trevors, J.T. and Paul, J.W. (1989). Carbon sources for bacterial denitrification. *Adv. Soil Sci*. 10, 113–142.

[12] Beeson, S. and Cook, M.C. (2004). Nitrate in groundwater: a water company perspective. *Q. J. Eng. Geol. Hydrogeol*. 37 (4), 261–270.

[13] Berger, L. (1989). Disappearance of amphibian larvae in the agricultural landscape. *Ecol. Int. Bull*. 17, 65–73.

[14] Birge, W.J., Westerman, A.G. and Spromberg, J.A. (2000). Comparative toxicology and risk assessment of amphibians. In: Sparling, D.W., Linder, G., Bishop, C.A. (Eds.), Ecotoxicology of Amphibians and Reptiles. SETAC, Pensacola (USA), pp. 727–792.

[15] Brender, J.D., Olive, J.M., Felkner, M., Suarez, L., Marckwardt, W. and Hendricks, K.A. (2004). Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. *Epidemiology* 15:330–336.

[16] Bruning-Fann, C.S. and Kaneene, J.B. (1993). The effects of nitrate, nitrite and N-nitroso compounds on human health: a review. *Vet Hum Toxicol*. 35:521–538.

[17] Burden, R.J. (1982). Nitrate contamination in New Zealand aquifers: a review. *N.Z. J. Sci*. 25 (3), 205–220.

[18] Carpenter, S.R. Caraco, N.F. Correll, D.L. Howarth, R.W. Sharpley, A.N. and Smith, V.H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl*. 8, 559–568.

[19] Carruthers, C. M. and Foster, P. M. (2005). Critical window of male reproductive tract

development in rats following gestational exposure to di-nbutyl phthalate. *Birth Defects Res. B. Dev. Reprod. Toxicol.* 74, 277–85.

[20] Cheng, S.Y. and Chen, J.C. (2002). Study on the oxyhemocyanin, deoxyhemocyanin, oxygen affinity and acid–base balance of Marsupenaeus japonicus following exposure to combined elevated nitrite and nitrate. *Aquatic Toxicol*. 61, 181–193.

[21] Conrad, J.J. (1990). Nitrate Pollution and Politics. Aldershot, Avebury.

[22] De Groef, B., Decallonne, B.R., Van der Geyten, S., Darras, V.M. and Bouillon, R. (2006). Perchlorate versus other environmental sodium/iodide symporter inhibitors: Potential thyroid-related health effects. *Eur. J. Endocrinol.* 155, 17–25.

[23] Doel, J.J., Benjamin, N., et al., (2005). Evaluation of bacterial nitrate reduction in the human oral cavity. *Eur. J. Oral Sci*. 113 (1), 14–19.

[24] Ema, M., Miyawaki, E., and Kawashima, K. (2000). Critical period for adverse effects on development of reproductive system in male offspring of rats given di-n-butyl phthalate during late pregnancy. *Toxicol. Lett*. 111, 271–278.

[25] Esciocak, S., Dundar, C., Basoglu, T., and Altaner, S. (2005). The effects of taking chronic nitrate by drinking water on thyroid functions and morphology. *Clin. Exp. Med*. 5, 66–71.

[26] Fan, A.M. and Steinberg, V.E. (1996). Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. *Regul. Toxicol. Pharm*. 23 (1), 35–43.

[27] Fan, A.M, Willhite, C.C. and Book, S.A. (1987). Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Regul Toxicol Pharmacol*. 7:135–148.

[28] Galloway, J.N. and Cowling, E.B. (2002). Reactive nitrogen and the world: 200 years of change. *Ambio*. 31:64–71.

[29] Gatseva, P., Vladeva, S. and Pavlov, K. (1998). Incidence of goiter among children in a village with nitrate contamination of drinking water. *Folia Medica* 40, 19–23.

[30] Gautam, S. and Iqbal, M. (2010). Using stable isotopes of nitrogen to study its source and transformation in a heavily farmed watershed. *Environ. Earth. Sci*. 60, 11–20.

[31] Grabda, E., Einszporn-Orecka, T., Felinska, C. and Zbanysek, R., (1974). Experimental methemoglobinemia in trout. *Acta Ichthyol. Piscat*. 4, 43–71.

[32] Gray, L. E., Jr., Ostby, J., Furr, J., Price, M., Veeramachaneni, D. N., and Parks, L. (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicol. Sci*. 58, 350–65.

[33] Gulis, G., Czompolyova, M. and Cerhan, J. R. (2002). An ecologic study of nitrate in municipal drinking water and cancer incidence in Tmava District, Slovakia. *Environmental Research*, 88, 182- 187.

[34] Gupta, S.K., Gupta, R.C., Chhabra, S.K., Eskiocak, S. and Gupta, A.B. (2008). Health issues related to N pollution in water and air. *Ind. Agric. Environ. Health*, 94: 1469-1477.

[35] Hansen, P.R., Taxvig, C., Christiansen, S., Axelstad, M., Boberg, J., Kiersgaard, M.K., Nellemann, C. and Hass, U. (2009). Evaluation of Endocrine Disrupting Effects of Nitrate after In Utero Exposure in Rats and of Nitrate and Nitrite in the H295R and T-Screen Assay. *Toxicological Sciences* 108(2), 437–444

[36] Harte, J., Holdren, C., Schneider, R. and Shirley, C. (1991). Toxics A to Z: A Guide to Everyday Pollution Hazards. University of California Press, Berkeley.

[37] Hecnar, S.J. (1995). Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. *Environ. Toxicol. Chem*. 14, 2131–2137.

[38] Horing, H. and Chapman, D. (2004). Nitrates and Nitrites in Drinking Water. In: World Health Organization Drinking Water Series. IWA Publishing, London.

[39] Hudak, P.F. and Blanchard, S. (1997). Land use and groundwater quality in the Trinity Group outcrop of north-central Texas, USA. *Environ Int* 23(4):507–517.

[40] Itoh, M., Takemon, Y., Makabe, A., Yoshimizu, C., Kohzu, A., Ohte, N., Tumurskh, D., Tayasu, I., Yoshida, N. and Nagata, T. (2011). Evaluation of wastewater nitrogen transformation in a natural wetland

(Ulaanbaatar, Mongolia) using dual-isotope analysis of nitrate. *Sci. Total Environ*. 409, 1530–1538.

[41] Jaffe, E.R. (1981). Methemoglobin pathophysiology. In: The Function of Red Blood Cells: Erythrocyte Pathobiology. Proceedings of an International Symposium on Erythrocyte Pathobiology. New York:Alan R. Liss, 133–151.

[42] Jain, CK, Bandyopadhyay, A. and Bhadra, A. (2009). Assessment of ground water quality for drinking purpose, District Nainital, Uttarakhand, India.*Environ Monit Assess Springer*, 166:663–676.

[43] Jensen, F.B. (1996). Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (Astacus astacus). *Aquatic Toxicol*. 34, 95–104.

[44] Johansson, M., Rsanen, K. and Merila, J. (2001). Comparison of nitrate tolerance between different populations of the common frog, Rana temporaria. *Aquatic Toxicol*. 54, 1–14.

[45] Knobelock, L., Salna, B., Hogan, A., Postle, J. & Anderson, H. (2000). Blue babies and nitrate-contaminated well water. Environ. *Health Perspect*., 108, 675–678.

[46] Knowles, R. (1982). Denitrification. Microbiol. Rev. 46 (1), 43–70.

[47] Kross, B.C., Hallberg, G.R., Bruner, D.R., Sherry, Holmes. K. and Johnson, J.K. (1993). The nitrate contamination of private well water in Iowa. *American J Public Health* 83(2):270–272.

[48] Leelavathi, C., U.KrishnaSainath, U.K. and Rabbani, A.K. (2016). Physicochemical characterization of ground water of Autonagar, Vijayawada, Krishna district.*IJEDR*. 4(2): 1324-1328.

[49] Li, J. Li, F.D. Liu, Q. and Suzuki, Y. (2014). Nitrate pollution and its transfer in surface water and groundwater in irrigated areas: A case study of the Piedmont of South Taihang Mountains, China. *Environ. Sci. Process. Impacts* 16, 2764–2773.

[50] Li, S.L., Liu, C.Q., Li, J., Xue, Z.C., Guan, J., Lang, Y.C., Ding, H. and Li, L.B. (2013). Evaluation of nitrate source in surface water of southwestern China based on stable isotopes. *Environ. Earth. Sci*. 68, 219–228.

[51] Liu, T., Wang, F., Michalski, G., Xia, X.H. and Liu, S.D. (2013). Using 15N, 17O, and 18O to determine nitrate sources in the Yellow River, China. *Environ. Sci. Technol*. 47, 13412–13421.

[52] Lui, J., Dorjderem, A., Fu, J., Lei, X., Lui, H., Macer, D., Qiao, Q., Sun, A., Tachiyama, K., Yu, L. and Zheng, Y. (2011). Water ethics and water resource management. Ethics and Climate Change in Asia and the Pacific (ECCAP) Project, Working Group 14 Report. UNESCO Bangkok.

[53] Lundberg, J.O., Weitzberg, E., et al., (2004). Nitrate, bacteria and human health. *Nat. Rev. Microbiol*. 2 (7), 593–602.

[54] Majumdar, D. (2003). The Blue Baby Syndrome. *Resonance*, 8, 20-30

[55] Manassaram, D.M., Backer, L.C. and Moll, D.M. (2006). A review of nitrates in drinking water: Maternal exposure and adverse reproductive and developmental outcomes. *Environ. Health Perspectives*. 114: 320-327.

[56] Marco, A., Quilchano, C. and Blaustein, A.R. (1999). Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. *Environ. Toxicol. Chem*. 18, 2836– 2839.

[57] Margat, J. (2008). Exploitations et utilisations des eaux souterraines dans le monde. Coédition: UNESCO et BRGM, 52p.

[58] Margat, J., and Van der Gun, J. (2013). Groundwater around the world: a geographic synopsis. CRC Press.

[59] Mason, C.F. (2002). Biology of freshwater pollution, Fourth Edition. Prentice Hall, Harlow, Essex.

[60] Mishra, R.K. and S.C. Dubey. (2015). Fresh water availability and its global challenge. *IJESIRD* 2(6):351-407.

[61] Mueller, B.A., Newton, K., Holly, E.A. and Preston-Martin, S. (2001). Residential water source and the risk of childhood brain tumors. *Environ. Health Perspectives*, 109, 551–556.

[62] NAS. (1977). Drinking Water and Health: Inorganic Solutes. Washington, DC: National Academy of Sciences, 411–439.

[63] Nash, L. (1993). Water quality and health. In: Gleik, P.H. (Ed.), Water in Crisis: A Guide to the Worlds Fresh Water Resources. Oxford University Press, New York, pp. 25–39.

[64] Oldham, R.S., Lathan, D.M., Hilton-Brown, D., Towns, M., Cooke, A.S. and Burn, A. (1997). The effect of ammonium nitrate fertilizer on frog (Rana temporaria) survival. *Agric. Ecosys. Environ*. 61, 69–74.

[65] Panesar, N. S. and Chan, K. W. (2000). Decreased steroid hormone synthesis from inorganic nitrite and nitrate: Studies in vitro and in vivo. *Toxicol. Appl. Pharmacol* 169, 222–30.

[66] Pant, N. and Srivastava, S. P. (2002). Testikular and spermatotoxic effect of nitrate in mice. *Hum. Exp. Toxicol*. 21, 37–41.

[67] Parks, L. G., Ostby, J. S., Lambright, C. R., Abbott, B. D., Klinefelter, G. R., Barlow, N. J., and Gray, L. E., Jr. (2000). The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicol. Sci.* 58, 339–349.

[68] Ray, C. and Schock, S.C. (1996). Comparability of large-scale studies of agricultural chemical contamination of rural private wells. *Ground Water Monit Remed* 16(2):92–102.

[69] Roth, A. C., Herkert, G. E., Bercz, J. P. and Smith, M. K. (1987). Evaluation of the developmental toxicity of sodium nitrite in Long– Evans rats. *Fundam. Appl. Toxicol.* **9,** 668–677.

[70] Roth, A. C. and Smith, M. K. (1988). Nitrite-induced iron deficiency in the neonatal rat. *Toxicol. Appl. Pharmacol.* **96,** 43–51.

[71] Safe Drinking Water Act of 1974. (1974). Public Law 42 U.S.C. s/s 300f. Available: http://www.epa.gov/region5/defs/html/ sdwa.htm

[72] Sandstedt, C.A. (1990). Nitrates: Sources and their Effects upon Humans and Livestock. American University, Washington, DC

[73] Schuytema, G.S. and Nebeker, A.V. (1999a). Comparative effects of ammonium and nitrate compounds on Pacific treefrog and African clawed frog embryos. Arch. *Environ. Contam. Toxicol.* 36, 200–206.

[74] Schuytema, G.S. and Nebeker, A.V. (1999b). Comparative toxicity of ammonium and nitrate compounds to Pacific treefrog and African clawed frog tadpoles. *Environ. Toxicol. Chem*. 18, 2251–2257.

[75] Scott, G. and Crunkilton, R.L. (2000). Acute and chronic toxicity of nitrate to fathead minnows (Pimephales promelas), Ceriodaphnia dubia and Daphnia magna. *Environ. Toxicol. Chem*. 19, 2918–2922.

[76] Spalding, R.F. and Exner, M.E. (1993). Occurrence of nitrate in groundwater: a review. *J. Environ. Qual.* 22, 392–402.

[77] Speijers, G.J.A. & Van den Brandt, P.A. (2003): Nitrate (and potential endogenous formation of N-nitroso compounds). [Online]. WHO Fd Additives Series 50. Available from: http://www.inchem.org/documents/jecfa/jecmono/v50je06.htm

[78] Spiegelhalder, B., Eisenbrand, G., et al., (1976). Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. *Food Cosmet. Toxicol*. 14, 545–548.

[79] Tajta´kova´, M., Semanova´, Z., Tomkova´, Z., Szo¨keova´, E., Majoros, J., Ra´dikova´, Z., Sebo¨kova´, E., Klimes, I., and Langer, P. (2006). Increased thyroid volume and frequency of thyroid disorders signs in schoolchildren from nitrate polluted area. *Chemosphere* 62, 559–64.

[80] Tesoriero, A. J., Liebscher, H. and Cox, S.E. (2000). Mechanism and rate of denitrification in an agricultural watershed: electron and mass balance along groundwater flow paths. *Water Resour. Res.* 36 (6), 1545–1559.

[81] Tsezou, A., Kitsiou-Tzeli, Galla, A., Gourgiotis, D., Mitrou, S., Molybdas, P. and Sinaniotis, C. (1996). High nitrate content in drinking water: Cytogenetic effects in exposed children. *Arch. Environ*. Health, 51, 458–461.

[82] US Environmental Protection Agency, (1986). Quality Criteria for Water. EPA 440/5-86-001, Washington, DC.

[83] Vladeva, S., Gatseva, P. and Gopina, G. (2000). Comparative analysis of results from studies of goitre in children from Bulgarian villages with nitrate pollution of drinking water in 1995 and 1998. Cent. *Eur. J. Public Health* 8, 179–181.

[84] van Maanen, J. M., van Dijk, A., Mulder, K., de Baets, M. H., Menheere, P. C., van

der Heide, D., Mertens, P. L., and Kleinjans, J. C. (1994). Consumption of drinking water with high nitrate levels causes hypertrophy of the thyroid. *Toxicol. Lett. Jun* 72, 365–74.

[85] Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H. and Tilman, D.G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl*. 7:737–50.

[86] Walker, R. (1996). The metabolism of dietary nitrites and nitrates. *Biochem. Soc. Trans*. 24 (3), 780–785.

[87] Ward, M. H., Cantor, K. P., Riley, D., Merkle, S. and Lynch, C. F. (2003). Nitrate in public water supplies and the risk of bladder cancer. *Epidemiology*, 14, 183-190.

[88] Ward, M.H., deKok, T.M., Levallois, P., Brender, J., Gulis, G., Nolan, B.T. and VanDerslice, J. (2005). Workgroup report: Drinking-water nitrate and health-recent findings and research needs. *Environ. Health Perspect.* 113, 1607–1614.

[89] Wederkinch, E. (1988). Population size, migration barriers, and other features of Rana dalmatina populations near Køge, Zealand, Denmark. Memo. *Soc. Fauna Flora Fenn*. 64, 101–103.

[90] Weyer, P.J., Cerhan, J.R., Kross, B.C., Hallberg, G.R., Kantamneni, J., Breuer, G., Jones, M.P., Zheng, W. and Lynch, C.F. (2001). Municipal drinking water nitrate level and cancer risk in older women: The Iowa women's health study. *Epidemiology*, 11, 327–338.

[91] Wogan, G.N., Generoso, W., Koller, L.D. and Smith, R.P. (1995). Nitrate and Nitrite in Drinking Water. Washington, DC: National Academic Press.

[92] Wolfe, A.H. and Patz, J.A. (2002). Reactive nitrogen and human health: acute and long-term implications. *Ambio* 31, 120– 125.

[93] WHO. (2007). Nitrate and nitrite in drinking water. Background document for development of WHO Guidelines for Drinking-water Quality.

[94] World Health Organisation, (1999). Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E & F N Spon, London.

[95] World Heath Organisation, (2004). Guidelines for drinking water quality, 3rd Edition. WHO, Geneva.

[96] Xu, Q. and Oldham, R.S. (1997). Lethal and sublethal effects of nitrogen fertilizer ammonium nitrate in common toad (Bufo bufo) tadpoles. *Arch. Environ. Contam. Toxicol*. 32, 298–303.

[97] Yang, C.Y., Wu, D.C. and Chang, C.C. (2007). Nitrate in drinking water and risk of death from colon cancer in Taiwan. *Environ. Int*., 33: 649-653.

[98] Yang, L. Song, X. Zhang, Y. Han, D. Zhang, B. and Long, D. (2012). Characterizing interactions between surface water and groundwater in the Jialu River basin using major ion chemistry and stable isotopes. *Hydrol. Earth Syst. Sci. Discuss*. 9, 5955–5981.

[99] Zaki, A., Ait Chaoui, A., Talibi, A., Derouiche, A. F., Aboussaouira, T., Zarrouck, K., Chait, A., and Himmi, T. (2004). Impact of nitrate intake in drinking water on the thyroid gland activity in male rat. *Toxicol. Lett*. 147, 27–33.