3. REVIEW OF LITERATURE

3.1. Heavy metals

3.1.1. Identification of heavy metals

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). "Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times or more, greater than water (Huton and Symon, 1986; Battarbee et al., 1988; Nriagu and Pacyna 1988; Nriagu, 1989; Garbarino et al., 1995, Hawkes, 1997). Heavy metals Occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed. To a small extent, they enter the body system through food, air, and water and bio-accumulate over a period of time. (Lenntech, 2004; UNEP/GPA, 2004). It is generally considered that heavy metals originate from two primary sources: natural inputs (e.g. parent material weathering) and anthropogenic inputs (e.g. metalliferous industries and mining vehicle exhaust, agronomic practices, etc.) (Zhang, 2006). Some heavy metals have bio-importance as trace elements but, the biotoxic effects of many of them in human biochemistry are of great concern. Hence, it is important to know sources of heavy metals, their concentration, oxidation states, leaching processes, chemical conversions and their modes of deposition to pollute the environment. When ingested, they combine with the body's biomolecules, like proteins and enzymes to form stable biotoxic compounds, thereby mutilating their structures and hindering them from the bioreactions of their functions (Duruibe et al., 2007)

3.1.2. Heavy metal emission

Heavy metals can be emitted into the environment by both natural and anthropogenic causes. The major causes of emission are the anthropogenic sources specifically mining operations (Hutton and Symon, 1986; Battarbee et al., 1988; Nriagu, 1989). Heavy metals are emitted both in elemental and compound (organic and inorganic) forms. Anthropogenic sources of emission are the various industrial point sources including former and present mining sites, foundries and smelters, combustion by-products and traffics (UNEP /GPA, 2004).

3.1.3. Heavy metal exposure

3.1.3.1. Human exposure through food, air and water

Heavy metal pollution of surface and underground water sources results in considerable soil pollution and pollution increases when mined ores are dumped on the ground surface for manual dressing (Garbarino et al., 1995; INECAR, 2000). Surface dumping exposes the metals to air and rain thereby generating much AMD. When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues (Trueby, 2003). Animals that graze on such contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues, and milk, if lactating (Habashi, 1992;

Garbarino et al., 1995; Horsfall and Spiff, 1999; Peplow, 1999). Humans are in turn exposed to heavy metals by consuming contaminated plants and animals, and this has been known to result in various biochemical disorders. In summary, all living organisms within a given ecosystem are variously contaminated along their cycles of food chain.

Table 1. United State Environmental Protection Agency (USEPA) maximum contamination levels for heavy metal concentration in air, soil and water.

Heavy metal	Max conc. in air (mg/m3)	Max. conc. in sludge (soil) (mg/ Kg or ppm	Max. conc. in drinking water (mg/l)	Max conc. in H20 supporting aquatic life (mg/l or ppm)
Cd	0.1-0.2	85	0.005	0.008
Pb	- -	420	0.01_ (0.0)	0.0058
Zn^2	1, 5	7500	5.00	0.0766
Hg	- -	<1	0.002	0.05
Ca	5	Tolerable	50	Tolerable >50
Ag	0.01	- -	0.0	0.1
As	- -	- -	0.01	- -

3.1.3.2. Human exposure through industrial products

Industrial products that are used in homes, and which have been produced with heavy metals are sources of human exposure to such heavy metals. Mercury exposure is through disinfectants (like mercurochrome), antifungal agents, toiletries, creams and organo-metallics (McCluggage, 1991). Cadmium exposure is through nickel/cadmium batteries and artist paints while lead exposure is through wine bottle wraps, mirror coatings, batteries, old paints and tiles and linolein amongst others. Infants are more susceptible to the endangering effects of exposure to heavy metals.

3.1.3.3. Occupational exposure

Heavy metal exposure occurs significantly by occupational exposure. Workers of the mining and production of cadmium, chromium, lead, mercury, gold and silver have been reported to be thus exposed; also inhabitants around industrial sites of heavy metal mining and processing, are exposed through air by suspended particulate matters (SPM) (Heyer, 1985; USDOL, 2004; Ogwuegbu and Muhanga, 2005).

Table 2. Recommended (Daily) Dietary Allowances (RDA) of The Food and Nutrition Board (Published by the National Academy of Science, Washington, DC, U.S.A).

	Age (Years)	Weight (kg)	Ca (mg)	Fe (mg)	Mg (mg)	Zn (mg)
Infants	0-1/2	6	360	10	60	3
	½-1	9	540	15	70	5
Children	1-3	13	800	15	150	10
	4-6	20	800	10	200	10
	7-10	30	800	10	250	10
Males	11-14	44	1200	18	350	15
	15-18	61	1200	18	400	15
	19+	67+	800	10	350	15
Females	11-18	44-54	1200	18	300	15
	19+	58	800	18(10)	300	15
Pregnant			1200	18+	450	20
Lactating			1200	15	450	25

3.1.4. Biological hazards of heavy metals

With the rapid development of urbanization and industrialization in recent years, heavy metal contamination has become a topic of extensive study with many reports in the recent literatures (Hu et al., 2006; Huang et al., 2006; Liu et al., 2006; Rodrigues et al., 2006; Shi et al., 2007; Chen et al., 2008). Heavy metals are of considerable environmental concerns due to their toxicity, wide sources, non-biodegradable properties and accumulative behaviors (YU et al., 2008). Metals, even at relatively low concentration, produce their toxic action by disturbing animal metabolism, altering hematology and blood chemistry and activating or inhibiting enzymes (Uehara et al., 1985; Szulinski and Szulinska, 1994 Karmakar et al., 2000; Adham et al., 2001, 2002; Adham, 2002;; Mugahi et al., 2003; Viegas-Crespo et al., 2003; El-Demerdash et al., 2004; Rogival et al., 2006; Swiergosz-Kowalewska et al., 2006; Sanchez-Chardi et al., 2008).

3.1.5. Heavy metal toxicity

3.1.5.1. Biochemistry of toxicity

The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes. When ingested, in the acid medium of the stomach, they are converted to their stable oxidation states (Zn2+, Pb2+, Cd2+, As2+, As3+, Hg2+ and Ag+) and combine with the body's biomolecules such as proteins and enzymes to form strong and stable chemical bonds. The equations below show their reactions during bond formation with the sulphydryl groups (-SH) of cysteine and sulphur atoms of methionine (-SCH3) (Ogwuegbu and Ijioma, 2003).

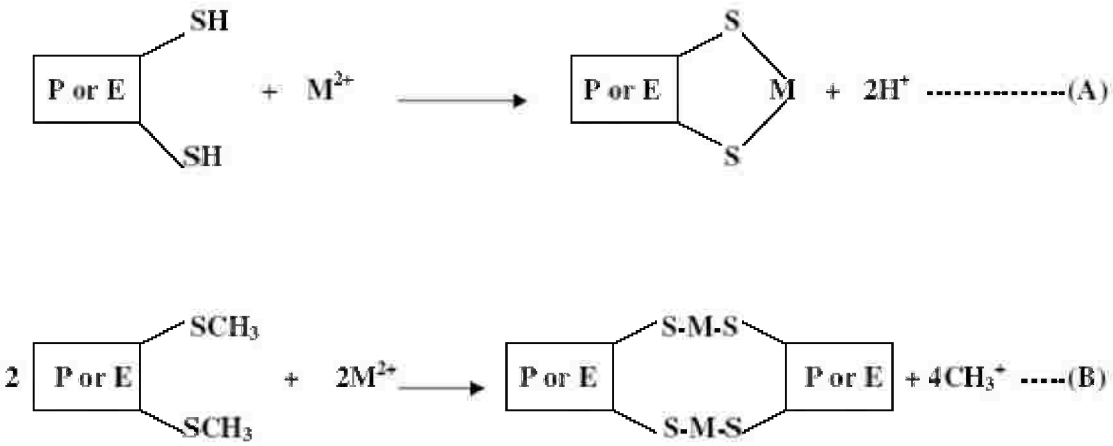

Fig. 1: Biochemistry of heavy metals toxicity

Where: (A) = Intramolecular bonding; (B) = Intermolecular

Bonding; P = Protein; E = Enzyme; M = Metal

The hydrogen atoms or the metal groups in the above case are replaced by the poisoning metal and the enzyme is thus inhibited from functioning,

Whereas the protein–metal compound acts as a substrate and reacts with a metabolic enzyme (Holum, 1983).

3.1.5.2. Mechanisms of metals toxicity

Metals generate many of their deleterious effects through the formation of free radicals, resulting in DNA damage, lipid peroxidation, depletion of protein, sulfhydryls (eg, glutathione), and other effects. These reactive radicals include a wide range of chemical species, including oxygen, carbon, and sulfur radicals originating from the superoxide radical, hydrogen peroxide, and lipid peroxides, and also from chelates of amino acids, peptides, and proteins complexed with the toxic metals (Valko et al., 2005).

3.2. Cobalt

3.2.1. Cobalt identity

Cobalt is a naturally occurring element found in rocks, soil, water, plants, and animals. There are radioactive and non radioactive forms of cobalt. Non radioactive cobalt, referred to as stable cobalt, is used to produce alloys (mixtures of metals) used in the manufacture of aircraft engines, magnets, grinding and cutting tools, artificial hip and knee joints. The two most commercially important radioactive cobalt isotopes are ^{60}Co (read as cobalt sixty), and ^{57}Co. ^{60}Co is used as a source of gamma rays for sterilizing medical equipment and consumer products, radiation therapy for treating cancer patients, for manufacturing plastics, and food irradiation. ^{57}Co is used in medical and scientific research. It takes about 5.7 years for half of ^{60}Co to give off its radiation and about 272 days for ^{57}Co; this period of time is called the half-life (ATSDR, 2001). Cobalt comprises 0.0025% of the weight of the earth's crust and is the 33rd most abundant element (Smith & Carson, 1981; Merian, 1985; Abbasi et al., 1989). Cobalt does not occur naturally as a base metal, but is a component of more than 70 naturally occurring minerals, including various sulfides, arsenides, sulfoarsenides, hydrates, and oxides. The most common cobalt minerals are the arsenide CoAs2–3 (smeltite), the arsenosulfide CoAsS (cobaltine), and the sulfide Co3S4 (linneite) (IARC, 1991). Normally, Co is absorbed from the stomach in the form of Vitamin B12 through interaction with an intrinsic factor and excreted via the urinary tract, maintaining a total body content of about 0.2 mg, which is equivalent to about 5mg of Vitamin B12 (Elinder and Friberg, 1986)

3.2.2. Sources and uses of cobalt

Sources of environmental cobalt are both natural and anthropogenic (Barceloux, 1999). Natural sources include erosion (wind-blown continental dusts), weathering of rocks and soil, seawater spray, volcanoes, forest fires, extraction by plants, and continental and marine biogenic emissions. The worldwide estimate for atmospheric cobalt emissions is 5350–6170 tonnes per year (Lantzy and Mackenzie, 1979; Nriagu, 1989). Cobalt compounds have been found to occur naturally in sea water, surface water, spring water, and groundwater (Smith and Carson, 1981). Cobalt is normally associated with copper or nickel; mined ore often contains only 0.1% elemental cobalt. About 44% of world production of cobalt comes from nickel ores. Cobalt is extracted from the metals in the ore by both flotation (sulfide ores) and gravity (arsenide ores); roasting or acid leaching is necessary to concentrate the cobalt (Barceloux, 1999). The major anthropogenic sources of environmental cobalt include mining and processing (smelting) of cobalt-bearing ores, the use of cobalt-containing sludge or phosphate fertilizers on soil, the disposal of cobalt-containing waste, and atmospheric deposition from activities such as the burning of fossil fuels and smelting and refining of metals (Smith and Carson, 1981).cobalt-

containing sewage sludge, phosphate fertilizers, processing of cobalt alloys, and industries that use or process cobalt compounds are estimated to emit an estimated 4000 tonnes per year of atmospheric cobalt (Lantzy and Mackenzie, 1979). Cobalt and its salts are widely used in industry as components in paints, grinding wheels, hygrometers and electroplating, varnishes, in vitamin B12, as a foam stabilizer in beer and as a catalyst in the petrochemical industry (Neve et al., 1991). Cobalt is also being used as a treatment of anemia and as illicit compound by athletes (Lippi et al., 2006). Cobalt compounds are also used to color glass, ceramics and used as a drier for porcelein enamel and paints (ATSDR, 2001).

- Human can be exposed to low levels of cobalt by breathing air, eating food, or drinking water. Food and drinking water are the largest sources of exposure to cobalt for the general population.
- Working in industries that makes or use cutting or grinding tools; mine, smelt, refine, or process cobalt metal or ores; or that produce cobalt alloys or use cobalt.
- The general population is rarely exposed to radioactive cobalt unless a person is undergoing radiation therapy. However, workers at nuclear facilities, irradiation facilities, or nuclear waste storage sites may be exposed to 60Co or 58Co (ATSDR, 2001).

3.2.3. Cobalt toxicity

Cobalt is suspected to be toxic to many cell types, including neural cells (Yang et al., 2004) and can induce cell death by apoptosis and necrosis (Huk et al., 2004). Cobalt can cause DNA fragmentation (Zou et al., 2001), activation of caspases (Zou et al., 2002) and increases production of reactive oxygen species (ROS) leading to oxidative stress (Olivieri et al., 2001). These free radicals may lead to cellular damage when the rate of their generation overcomes the rate of their decomposition by antioxidant defense systems, such as superoxide dismutase (SOD), catalase (CAT), or reduced GSH (Di Mascio et al., 1991; Mates et al., 1999; Datta et al., 2000).

3.2.3.1. Genotoxicity and related end-points

There are no available studies on genotoxic effects in animals exposed by inhalation. Male Swiss mice administered a single oral dose of cobalt (as cobalt chloride) at 0, 4.96, 9.92, or 19.8 mg/kg body weight exhibited a dose–response increase in percentages of chromosomal breaks and chromosomal aberrations in bone marrow cells (Palit et al., 1991a, 1991b, 1991c, 1991d). A single intraperitoneal injection of cobalt (as cobalt (II) chloride) at 12.4 or 22.3 but not 6.19 mg/kg body weight in BALB/c mice caused an increase in micronucleus formation after 30 h (Suzuki et al., 1993). F344 rats injected intraperitoneally with cobalt at 3 or 6 mg/kg body weight exhibited increased levels of oxidatively damaged DNA bases in the liver, kidney, and lung at 2 and 10 days following injection (Kasprzak et al., 1994). In mammalian test systems, many cobalt compounds and metals are genotoxic. Cobalt compounds and cobalt metals have been reported to cause clastogenic effects in mammalian cells such as human lymphocytes (Painter and Howard, 1982; Hamilton-Koch et al., 1986; Anard et al., 1997), transformation in hamster cells (Costa et al., 1982), sister chromatid exchanges in human lymphocytes (Andersen, 1983), and micronucleus formation in mouse bone marrow cells (Suzuki et al., 1993), human lymphocytes (Capomazza and Botta, 1991; Olivero et al. 1995; van Goethem et al., 1997), and rat type II epithelial lung cells (DeBoeck et al., 2003). Cobalt particles are genotoxic in vitro in human peripheral blood mononucleated cells (Anard et al., 1997; van Goethem et

al., 1997; De Boeck et al., 1998, 2003). In general, hard cobalt metal is more genotoxic than other cobalt compounds in vitro test systems.

3.2.3.2. Reproductive toxicity

3.2.3.2.1. Effects on fertility

Both rats exposed to cobalt (as cobalt chloride) at 13.3–58.9 mg/kg body weight per day for 2–3 months in drinking-water or diet (Nation et al., 1983; Domingo et al., 1984; Corrier et al., 1985; Mollenhauer et al., 1985; Pedigo et al., 1988; Pedigo and Vernon, 1993) and mice exposed to cobalt (as cobalt chloride) at 43.4 mg/kg body weight per day for 13 weeks in drinking-water exhibited testicular degeneration and atrophy (Anderson et al., 1992, 1993). In an abstract reported by Elbetieha et al. (2004), sexually mature male mice exposed to cobalt (II) chloride at 200, 400, or 800 mg/l in their drinking-water for 12 weeks were assessed for effects on fertility by breeding these exposed males to unexposed females. Fertility, as measured by successful matings, was reduced in mice exposed to cobalt chloride at 400 and 800 mg/l (internal doses of 46.91 ± 4.78 and 93.01 ± 6.76 mg/kg bodyweight per day, respectively). The number of implantation sites was significantly reduced in females mated with exposed males at 400 and 800 mg/l. The number of viable fetuses was decreased in females mated with males at all three exposure levels. In the 800 mg/l males, absolute epididymal weight was significantly decreased, whereas relative and absolute testes weights were decreased in males exposed to 400 and 800 mg/l. Epididymal sperm count was decreased in males of all three exposure levels. At 400 and 800 mg/l, males also exhibited reduced testicular sperm counts and daily sperm production. The testes displayed severe abnormalities including hypertrophy of the interstitial Leydig cells, congested blood vessels, degeneration of the spermatogonial cells, and necrosis of seminiferous tubules and interstitial tissue. In a study in which B6C3F1 mice were exposed by inhalation to cobalt sulfate heptahydrate (0, 0.3, 1, 3, 10, and 30 mg/m^3; equivalent to cobalt concentrations of 0, 0.11, 0.38, 1.14, 3.80, and 11.38 mg/m^3) for 6 h/day, 5 days/week, for 13 weeks, testicular atrophy in males and increased estrous cycle length in females were observed at 30 mg/m3. Sperm motility was decreased in mice exposed to 3 mg/m^3 or higher (lower exposures not assessed), and increased abnormal sperm and decreased testis and epididymal weights were observed in mice exposed to 30 mg/m^3 (Bucher et al., 1990; NTP, 1991)

3.2.3.2.2. Developmental toxicity

Oral exposure of female rats to cobalt (as cobalt chloride) at doses of 5.4 or 21.8 mg/kg body weight per day from gestation day 14 to lactation day 21 caused newborn pups to exhibit stunted growth and decreased survival. However, these effects occurred at exposures that also caused maternal toxicity, such as reduced body weight, reduced food consumption, and altered haematological measurements. No teratogenic effects were observed (Domingo et al., 1985). Another study reported that exposure of pregnant rats to cobalt (as cobalt sulfate) at 0–38 mg/kg body weight per day did not affect fetal death rates, maternal body weight gain, average litter size, or average fetal and placental weights. However, a dose-related increase was noted in the percentage of fetuses with retarded body weights (Szakmary et al., 2001). In contrast, Paternain et al. (1988) found no effects on fetal growth or survival after exposing rats to cobalt (as cobalt chloride) at 24.8 mg/kg body weight per day during gestation days 6–15. Exposure of pregnant mice to cobalt (as cobalt sulfate) at 19 mg/kg body weight per day also did not affect litter size,

postimplantation loss, or average fetal and placental weights (Szakmary et al., 2001). Rabbits exposed to cobalt (as cobalt sulfate) at doses of ≥38 mg/kg body weight per day exhibited complete maternal lethality and fetal loss. At 7.6 mg/kg body weight per day, rabbits had increased mortality, fetal resorption, and number of fetuses with retarded body weight (Szakmary et al., 2001).

3.2.3.3. Cobalt and respiratory effects

The effects of chronic occupational exposure to cobalt and cobalt compounds on the respiratory system in humans are well-documented. These effects include respiratory irritation, diminished pulmonary function, wheezing, asthma, pneumonia, and fibrosis and occurred at exposure levels ranging from 0.007 to 0.893 mg cobalt/m^3 (exposure from 2 to 17 years) (Anttila et al. 1986; Davison et al. 1983; Demedts et al. 1984a, 1984b; Deng et al. 1991; Gennart and Lauwerys 1990; Gheysens et al. 1985; Hartung et al. 1982; Kusaka et al. 1986a, 1986b, 1996a, 1996b; Nemery et al. 1992; Raffn et al. 1988; Rastogi et al. 1991; Ruokonen et al. 1996; Shirakawa et al. 1988, 1989; Sprince et al. 1988; Sundaram et al. 2001; Swennen et al. 1993; Tabatowski et al. 1988; Van Cutsem et al. 1987; Zanelli et al. 1994). These effects have been observed in workers employed in cobalt refineries, as well as hard metal workers, diamond polishers, and ceramic dish painters (painting with cobalt blue dye). Kusaka et al. (1986b) described an acute exposure of 15 healthy young men to atmospheres of hard metal dust containing 0.038 mg cobalt/m3 for 6 hours. Forced vital capacity (FVC) was reduced, but no dose response relation could be discerned. By contrast, 42 workers occupationally exposed to hard metal showed no decrease in ventilatory function at 0.085 mg cobalt/m^3, but significant changes in FEV1 (forced expiratory volume in 1 second) at 0.126 mg cobalt/m^3 (Kusaka et al. 1986b). Several other studies of hard metal workers have shown respiratory effects, including decreased ventilatory function, wheezing, asthma, and fibrosis (Kusaka et al. 1996a, 1996b; Ruokonen et al. 1996; Zanelli et al. 1994).

Swennen et al. (1993) performed a cross-sectional study on 82 workers in a cobalt refinery. Workers were examined for cobalt in blood and urine, a number of erythropoietic variables, thyroid metabolism, pulmonary function, skin lesions, and several serum enzymes. The concentrations of cobalt in blood and in urine after the shift were significantly correlated with those in air. Workers exposed to airborne cobalt metal, salts, or oxides (mean concentration 0.125 mg/m^3, range 0.001–7.7 mg/m^3) showed an increased ($p<0.05$) prevalence of dyspnea and wheezing and had significantly more skin lesions (eczema, erythema) than control workers. A dose-effect relation was found between the reduction of the FEV1 and the intensity of the current exposure to cobalt, as assessed by measurement of cobalt in blood, air, or urine. Gennart and Lauwerys (1990) examined the ventilatory functions of 48 diamond polishing workers, relative to 23 control workers. Exposure occurred mainly in one of two rooms, with mean airborne concentrations of 0.0152 and 0.1355 mg cobalt/m3; control subjects worked in other areas of the facilities, where no exposure to cobalt occurred. Significant decreases in ventilatory function were found in the exposed workers relative to the control workers. Duration of exposure played a significant factor, with no significant differences in workers who had been exposed for ≤5 years; reported decreases in ventilatory function were noted in workers exposed for > 5 years. Inhalation exposure to cobalt salts (exposure levels not reported) among glass bangle workers resulted in decreases in decreased ventilatory function, generally restrictive in nature, relative to controls (Rastogi et al. 1991).

Exposures of animals to cobalt-containing aerosols have resulted in pronounced respiratory effects. Animals exposed to aerosols of cobalt oxides and cobalt sulfate developed respiratory effects that varied in severity with exposure level and duration. A single 30-minute exposure of rats to relatively high levels (26–236 mg cobalt/m^3 as cobalt hydrocarbonyl) resulted in congestion, edema, and hemorrhage of the lung (Palmes et al. 1959). Prolonged exposure (3–4 months) of rats and rabbits to mixed cobalt oxides (0.4–9 mg cobalt/m^3) resulted in lesions in the alveolar region of the respiratory tract characterized histologically by nodular accumulation of Type II epithelial cells, accumulations of enlarged highly vacuolated macrophages, interstitial inflammation, and fibrosis (Johansson et al. 1984, 1987, 1991, 1992; Kyono et al. 1992; Palmes et al. 1959). Necrosis and inflammation of the respiratory tract epithelium (nasal turbinates, larynx, trachea, and bronchioles) were reported in rats exposed to 19 mg cobalt/m3 and mice exposed to 1.9 mg cobalt/m^3 or greater as cobalt sulfate over 16 days (Bucher et al. 1990; NTP 1991). Exposure of rats and mice to cobalt as cobalt sulfate for 13 weeks resulted in adverse effects on all parts of the respiratory tract, with the larynx being the most sensitive part (Bucher et al. 1990; NTP 1991).

3.2.3.4. Gastrointestinal Effects

3.2.3.4.1. Oral exposure

The first signs of the beer-cobalt cardiomyopathy syndrome were gastrointestinal effects and included nausea, vomiting, and diarrhea (Morin et al. 1971). Signs of heart failure subsequently appeared. These individuals had ingested an average of 0.04 mg cobalt/kg/day for a period of years during which cobalt sulfate was added to beer as a foam stabilizer; however, it is likely that alcohol consumption was also a factor. In pregnant women given cobalt supplements (alone or combined with iron) to prevent the decrease in hematocrit and hemoglobin levels commonly found during pregnancy (n=78), a small percentage of those treated complained of gastric intolerance (Holly 1955). No morphological changes in the gastrointestinal system were observed following exposure of 20 male rats for 3 months to 30.2 mg cobalt/kg/day as cobalt chloride in the drinking water (Domingo et al. 1984) or exposure for 4 months to 18 mg cobalt/kg/day as cobalt chloride by gavage (Holly 1955).

3.2.3.5. Hematological Effects

3.2.3.5.1. Inhalation exposure

Swennen et al. (1993) reported slightly, but statistically significantly, decreased levels of red cells and total hemoglobin (~4–5% decreases) in a group of 82 workers occupationally exposed to a mean concentration of 0.125 mg cobalt/m^3 as cobalt metal dust. No other studies were located regarding hematological effects in humans after inhalation exposure to cobalt. Increased levels of hemoglobin and increased numbers of basophils and monocytes have been observed in rats and guinea pigs, but not in dogs, exposed to 9 mg cobalt/m^3 as cobalt hydrocarbonyl for 3 months (Palmes et al. 1959). Polycythemia was reported in rats, but not mice, exposed to 1.14 mg cobalt/m^3 as cobalt sulfate for 13 weeks (Bucher et al. 1990; NTP 1991).

3.2.3.5.2. Oral exposure

Cobalt has been shown to stimulate the production of red blood cells in humans. Davis and Fields (1958) exposed six apparently normal men, ages 20–47, to a daily dose of cobalt chloride, administered as a 2% solution diluted in either water or milk, for up to 22 days. Five of the six received 150 mg cobalt chloride per day for the entire exposure period, while the sixth was started on 120 mg/day and later increased to 150 mg/day. Blood samples were obtained daily from free-flowing punctures of fingertips at least 2 hours after eating, and at least 15 hours after the last dosage of cobalt. Blood was analyzed for red blood cell counts, hemoglobin percentage, leukocyte counts, reticulocyte percentages, and thrombocyte counts. Exposure to cobalt resulted in the development of polycythemia in all six subjects, with increases in red blood cell numbers ranging from 0.5 to 1.19 million (~16–20% increase above pretreatment levels). Polycythemic erythrocyte counts returned to normal 9–15 days after cessation of cobalt administration. Hemoglobin levels were also increased by cobalt treatment, though to a lesser extent than the erythrocyte values, with increases of 6–11% over pretreatment values. In five of the six subjects, reticulocyte levels were elevated, reaching at least twice the pre-experiment values. Thrombocyte and total leukocyte counts did not deviate significantly from pretreatment values.

3.2.3.6. Body Weight Effects

3.2.3.6.1. Inhalation exposure

Weight loss, measured individually from time of initial examination throughout followup, was observed in a group of five diamond polishers suffering from cobalt-induced interstitial lung disease (Demedts et al. 1984b), but the exposure level of cobalt was not reported. Decreased body weight, relative to controls at study termination, was reported in both rats and mice exposed to 19 mg cobalt/m^3 as cobalt sulfate over 16 days or to 11.4 mg cobalt/m^3 for 13 weeks (Bucher et al. 1990; NTP 1991). A 13-week exposure to 11.4 mg cobalt /m^3 resulted in ruffled fur in male rats, with no clinical signs reported in female rats or either sex of mice (Bucher et al. 1990; NTP 1991). Chronic exposure of rats and mice to up to 1.14 mg cobalt/m^3 did not result in decreased body weight (Bucher et al. 1999; NTP 1998).

3.2.3.6.2. Oral exposure

No effects on body weight in animals were found following longer-term (1–5 months) exposure of rats to 10–30.2 mg cobalt/kg/day as cobalt chloride (Bourg et al. 1985; Domingo et al. 1984; Murdock 1959) or of guinea pigs to 20 mg cobalt/kg/day as cobalt sulfate (Mohiuddin et al. 1970). A significant decrease (33%) in body weight gain was observed following 8 weeks of exposure of rats to 4.2 mg cobalt/kg/day as cobalt sulfate (Clyne et al. 1988).

3.2.3.7. Neurological effects

3.2.3.7.1. Inhalation exposure

Occupational exposure to cobalt in humans has been reported to cause several effects on the nervous system, including memory loss (Wechsler Memory Scale-Revised), nerve deafness, and a decreased visual acuity (Jordan et al. 1990; Meecham and Humphrey 1991). Congestion in the vessels of the brain/meninges was reported in rats and mice

exposed to 19 mg cobalt/m^3 or greater as cobalt sulfate over 16 days (Bucher et al. 1990; NTP 1991).

3.2.3.7.2. Oral exposure

No studies were located regarding neurological effects in humans after oral exposure to stable cobalt. Several rodent studies have identified neurological effects following cobalt exposure. In Wistar rats, a single gavage dose of 4.25 mg cobalt/kg as cobalt chloride resulted in a moderate reduction in spontaneous activity, muscle tone, touch response, and respiration, while 19.4 mg cobalt/kg as cobalt sulfate caused a mild reduction the same parameters (Singh and Junnarkar 1991). In rats exposed to 4.96 mg cobalt/kg/day as cobalt chloride for 30 days in the drinking water, cobalt led to changes in sympathetically mediated contractile activity of isolated rat vas deferens (Mutafova-Yambolieva et al. 1994). Rats exposed to 6.44 mg cobalt/kg/day as cobalt nitrate in the drinking water showed an increased sensitivity and decreased maximal response to a cholinergic agonist (Vassilev et al. 1993). In rats exposed to 20 mg cobalt/kg/day as cobalt chloride for 57 days in the drinking water, cobalt enhanced behavioral reactivity to stress (the animals were less likely to descend from a safe platform to an electrified grid) (Bourg et al. 1985). Rats exposed to the same dose in the diet for 69 days showed a slower rate of lever pressing than controls, but no change in behavioral reactivity to stress (Nation et al. 1983). Longer-term exposure of rats to cobalt chloride (7 months) resulted in a significant increase in the latent reflex period at ≥0.5 mg cobalt/kg as cobalt chloride and a pronounced neurotropic effect (disturbed conditioned reflexes) at 2.5 mg cobalt/kg (Krasovskii and Fridlyand 1971).

3.2.3.8. Other toxicity

Dermal exposures on 3 consecutive days to cobalt (II) chloride (in dimethylsulfoxide) caused an increase in cellular proliferation in the lymph node assay in mice (10.8, 27, or 54.1 mg of cobalt per kilogram body weight per day), rats (9.6 or 19.2 mg of cobalt per kilogram body weight per day), and guinea-pigs (14.7 mg of cobalt per kilogram body weight per day) (Ikarashi et al., 1992a, 1992b).

3.2.4. Mechanism of cobalt toxicity

The biochemical mechanisms that underline cobalt toxicity in early postnatal periods are poorly understood. It has been suggested that oxidative stress was one of the main mechanisms involved in cobalt toxicity leading to the generation of reactive oxygen species (ROS), which in turn cause lipid peroxidation and protein oxidation in several tissues including the kidney (Ahmed and Siddiqui, 2007).

3.2.5. Effect of cobalt on human health

Cobalt has both beneficial and harmful effects on human health. Cobalt is beneficial for humans because it is part of vitamin B12 (ATSDR, 2001). Cobalt, an essential nutrient, serves at low doses as a cofactor for the activation of several enzymes, the formation of vitamin B12 and other cobalamines (Lee and Herbert, 1999). VitaminB12 is necessary for the organism, because it is involved in the formation of some proteins and in the normal functionality of the nervous system (Karovic et al., 2006). Exposure to high levels of cobalt can result in lung and heart effects and dermatitis. Liver and kidney effects have

also been observed in animals exposed to high levels of cobalt. Exposure to large amounts of radioactive cobalt or the radiation it emits can damage cells in human body. human body might also experience acute radiation syndrome which includes nausea, vomiting, diarrhea, bleeding, coma, and even death (ATSDR, 2001).

3.2.6. Cobalt and cancer

Non radioactive cobalt has not been found to cause cancer in humans or animals following exposure in food or water. Cancer has been shown, however, in animals who breathed cobalt or when cobalt was placed directly into the muscle or under the skin. Based on the laboratory animal data, the International Agency for Research on Cancer (IARC) has determined that cobalt and cobalt compounds are possibly carcinogenic to humans. Exposure to high levels of cobalt radiation can cause changes in the genetic materials within cells and may result in the development of some types of cancer (ATSDR, 2001).

3.2.7. Exposure to cobalt compounds

3.2.7.1. Short term exposure

Rats and mice exposed by inhalation to cobalt sulfate heptahydrate at cobalt concentrations of 19 and 1.9 mg/m^3, respectively, for 16 days exhibited necrosis and inflammation of the respiratory tract epithelium. Rats also developed thymus necrosis and testicular atrophy (Bucher et al., 1990; NTP, 1991). Male CFY rats exposed orally to cobalt chloride at 50 mg/kg body weight per day (equivalent to 12.4 mg of cobalt per kilogram body weight per day) for 3 weeks and co-exposed to drinking-water that contained 10% ethanol and 5% sugar exhibited cardiac damage that presented as incipient, multifocal myocytolysis with degeneration of myofibrils (Morvai et al., 1993). Rats exposed to ultrafine cobalt particles (diameter 20 nm) at concentrations of 2.72 mg/m^3 for 5 h or 2.12 mg/m3 for 5 h/day for 4 days displayed focal hypertrophy or proliferation of lower airway epithelium, macrophage damage, intracellular oedema of type I alveolar epithelium, interstitial oedema, and proliferation of type II alveolar epithelium (Kyono et al., 1992).

3.2.7.2. Medium-term exposure

Rats (strain not specified), guinea-pigs (strain not specified), and beagle dogs exposed to Cobalt (as cobalt hydrocarbonyl) at 9 mg/m^3 for 6 h/day, 5 days/week, for 3 months displayed foam cell aggregates (Palmes et al., 1959). These foam cell aggregates were composed of nodules of large macrophages with foamy cytoplasm, accompanied by moderate interstitial and peribronchial fibrosis, mild emphysema, and moderate peribronchial lymphoid hyperplasia. These aggregates were not present when animals were sacrificed and evaluated at 3 months or 6 months post-exposure. Rabbits exposed by inhalation for 1–4 months to cobalt chloride (0.4–2 mg/m^3) exhibited lesions of the alveolar region of the respiratory tract that were characterized by nodular accumulation of Type II epithelial cells and interstitial inflammation (Johansson et al., 1984, 1987, 1991, 1992). F344/N rats and B6C3F1 mice exposed by inhalation to cobalt sulfate heptahydrate (0, 0.3, 1, 3, 10, and 30 mg/m^3; equivalent to cobalt concentrations of 0, 0.11, 0.38, 1.14, 3.80, and 11.38 mg/m^3) for 6 h/day, 5 days/week, for 13 weeks developed adverse effects through out the respiratory tract (Bucher et al., 1990; NTP, 1991). At concentrations ≥0.3 mg/m^3 (cobalt concentrations ≥0.11 mg/m^3), both rats and mice developed squamous

metaplasia of the larynx (the most sensitive tissue), such that a NOAEC could not be determined. Rats developed chronic inflammation of the larynx at≥1 mg/m^3 and more severe effects in the nose, larynx, and lung at higher exposures. Mice exhibited acute inflammation of the nose at ≥3 mg/m^3 and more severe effects in the nose, larynx, and lung at higher exposures. At 30 mg/m^3, mice exhibited hyperplasia of the mediastinal lymph nodes and testicular atrophy and increased estrous cycle length in females. Both rats and mice exhibited histiocytic infiltrates of the lung at similar exposure levels. Sperm motility was decreased in mice exposed to 3 mg/m^3 or higher (lower exposures not assessed), and increased abnormal sperm and decreased testis and epididymal weights were observed in mice exposed to 30 mg/m^3. Rats exposed for 2–3 months to cobalt at 26–30.2 mg/kg body weight per day in the diet (as cobalt sulfate) or in drinking-water (as cobalt chloride) exhibited increased heart weight and degenerative heart lesions (Grice et al., 1969; Domingo et al., 1984). Rats exposed to cobalt (as cobalt sulfate) in the diet at 8.4 mg/kg body weight per day for 24 weeks experienced significant reductions in cardiac enzyme activity levels, such as manganese superoxide dismutase, succinate cytochrome c oxidase, NADH cytochrome c reductase, and cytochrome c oxidase, and a reduction in mitochondrial ATP production (Clyne et al., 2001). Rats exposed to cobalt (as cobalt chloride) at 10–18 mg/kg body weight per day for 4–5 months exhibited renal injury, such as histological alteration of proximal tubules (Holly, 1955; Murdock 1959).

3.2.7.3. Long-term exposure and carcinogenicity

A study by the NTP examined the carcinogenicity of cobalt by inhalation in mice (NTP, 1998; Bucher et al., 1999). Groups of 50 male and 50 female B6C3F1 mice were exposed to cobalt sulfate heptahydrate at 0, 0.3, 1, or 3 mg/m^3 for 6 h/day, 5 days/week, for 105 weeks. Cobalt concentrations in this study were 0, 0.11, 0.38, 1.14, and 3.80 mg/m^3. Mean body weights were increased in all treated females and decreased only in the high-dose males. Survival was not adversely affected by treatment. The incidences of benign and malignant alveolar/bronchiolar neoplasms were increased in a concentration-dependent manner: males, 11/50, 14/50, 19/50, and 28/50 for 0, 0.3, 1, and 3 mg/m^3, respectively; females, 4/50, 7/50, 13/50, and 18/50 for 0, 0.3, 1, and 3mg/m^3, respectively. There were no increased incidences of neoplasms in other tissues. The NTP concluded that there was clear evidence of carcinogenic activity. Another study by the NTP examined the carcinogenicity of cobalt by inhalation in rats (NTP, 1998; Bucher et al., 1999). Groups of 50 male and 50 female Fischer 344/N rats were exposed to cobalt sulfate heptahydrate at 0, 0.3, 1, and 3 mg/m^3 (equivalent to cobalt concentrations of 0, 0.11, 0.38, 1.14, and 3.80 mg/m^3) for 6 h/day, 5 days/week, for 105 weeks. Mean body weights and survival were unaffected by treatment. Rats exhibited a concentration-related increase in the incidence of benign and malignant alveolar/bronchiolar neoplasms in male and female rats and benign and malignant pheochromocytomas in female rats. The incidences of benign and malignant alveolar/bronchiolar neoplasms were 1/50, 4/50, 4/48, and 7/50 for 0, 0.3, 1, and 3 mg/m^3, respectively, in males and 0/50, 3/49, 16/50, and 16/50 for 0, 0.3, 1, and 3 mg/m^3, respectively, in females. Although many of the alveolar/bronchiolar lesions were morphologically similar to those that arise spontaneously, the lesions in rats, unlike those in mice, were predominantly fibrotic, squamous, or mixtures of alveolar/bronchiolar epithelium and squamous or fibrous components. Squamous metaplasia of alveolar/bronchiolar epithelium, which is a common response to pulmonary injury, was observed in a number of rats. In females, incidences of benign and malignant pheochromocytomas of the adrenal medulla were 2/48, 1/49, 4/50, and 10/48 for 0, 0.3, 1,

and 3 mg/m^3, respectively. In males, the incidences of benign and malignant pheochromocytomas of the adrenal medulla were 15/50, 19/50, 25/50, and 20/50 for 0, 0.3, 1, and 3 mg/m^3, respectively. Pheochromocytomas are common spontaneous neoplasms in male Fischer F344/N rats, but have a lower spontaneous occurrence in females. There were no increased incidences of neoplasms in other tissues. The NTP concluded that there was some evidence of carcinogenic activity in male rats, but clear evidence in female rats. Heath reported that (1954, 1956, 1960) treated groups of 10 male and 10 female hooded rats with a single intramuscular injection of 28 mg of cobalt metal powder. The cobalt metal particles ranged in size from 3.5 μm × 3.5 μm to 17 μm × 12 μm, with large numbers of long narrow particles 10 μm × 4 μm. The rats were injected in the thigh. The observation period was 122 weeks, during which 4/10 male and 5/10 female rats developed sarcomas, mostly rhabdomyosarcomas, at the injection site. In a related study, 80 female hooded rats (divided into three groups of 16, 14, and 50) were intramuscularly injected with 28 mg of wear particles (ground artificial hip or knee prostheses composed of a cobalt–chromium–molybdenum alloy) (Heath et al., 1971; Swanson et al., 1973). No control group was reported. Animals were observed for up to 29 months. The incidences of sarcomas at the injection site were 3/16, 4/14, and 16/50. Half the tumours were rhabdomyosarcomas, and the remainder were fibrosarcomas. In a related study, Heath & Daniel (1962) injected two groups of 10 female hooded rats with 28 mg cobalt metal powder (3.5 μm × 3.5 μm to 17 μm × 12 μm, with large numbers of long narrow particles 10 μm × 4 μm) through the right dome of the diaphragm (first group) or through the fourth left intercostal space (second group). Animals were observed for up to28 months. Of the diaphragm-treated rats, 6/10 died within 3 days, and in the rats injected through the intercostal space, 2/10 died within 3 days. Of the 12 rats that survived the injection, 4 developed intrathoracic sarcomas. Three of these sarcomas were of mixed origin and included rhabdomyosarcomatous elements, while the fourth rhabdomyosarcoma arose in the intercostal muscle. (Jasmin and Riopelle., 1976) injected groups of 20 and 18 female Sprague-Dawley rats with 5 mg of metallic cobalt powder or cobalt sulfide powder, respectively, into each pole of the right kidney. After 12 months, necropsies were conducted, and no tumours were observed in the kidneys of treated or control rats.

3.3. Chelation therapy

3.3.1. Chelation therapy identity

Chelation therapy is an established treatment for the removal of metal toxins by converting them to a chemically inert form which can be excreted in the urine (Current procedural terminology, 2012). Chelation therapy is thought to not only remove contaminating metals but also to decrease free radical production (Lamas and Ackermann, 2000).

3.3.2. Chelating agents

3.3.2.1. Chelating agents identity

Chelating agents are organic compounds capable of binding metal ions to form complex ring-like structure called 'chelates'. Chelating agents possess "ligand" binding atoms that form either two covalent linkages or one covalent and one co-ordinate or two co-ordinate linkages in the case of bidentate chelates. Mainly atoms like S, N and O function as ligating atoms in the form of chemical groups like –SH, –S-S, –NH2, =NH, –

OH, $-OPO_3H$, or >C=O. Bidentate or multidentate ligands form ring structures that include the metal ion and the two-ligand atoms attached to the metal (Andersen,1999). Multidentate ligands which occupy more of the coordination positions of a metal ion will generally (but not always) give a complex of greater stability than otherwise. Similarly, whereas the net ionic charge of the chelator defines its absorption, distribution and ability to reach the metal ion for binding; the net ionic charge of the complex decides its elimination from the specific site and excretion from the body. Thus, it is important that a chelator satisfy certain criteria that allow it to:

(1) Transport across physiological barriers into compartments where a toxic metal ion is concentrated.
(2) Form a stable complex with the metal after removing it from the biological chelator, if required at the site.
(3) Form a chelation complex whose properties render it non-toxic and facilitate its excretion, not only from the site of deposition, but also from the body (Jones, 1994).

3.3.2.2. Characteristics of an Ideal Chelator

1. Greater Affinity, Low Toxicity
2. Ability to compete with natural chelators
3. Ability to penetrate cell membranes
4. Rapid elimination of the toxic metal
5. High water solubility
6. Capacity to form non-toxic complexes
7. Same distribution as the metal
8. Resistance to biotransformation
9. Ability to retain chelating ability at the pH of body fluid (Baum, 1999; Guldager et al., 1996; Fournier et al., 1988).

3.3.2.3. Chelating agents used for treatment of Cobalt toxicity

- **EDTA**
- **Cyclam**

3.3.2.3.1. EDTA (ethylene diamine tetraacetic acid)

Ethylene diamine tetraacetic acid, here after will be abbreviated EDTA ,is a chelation therapy which often used for treatment aimed at reducing calcium deposits, removing heavy metals, controlling lipid peroxidation resulting from free radical pathology, and reducing platelet aggregation in the clinical management of atherosclerosis and related disorders (Halstead, 1979). EDTA is a polydentate ligand. More specially is a hexadentate ligand which has six teeth or binding points to the central metal atoms, Thus EDTA which is N_2O_4 chromophoric type ligand. This ligand can bind to metal ions through the two nitrogens atoms and the four oxygen atoms forming metal complexes. These complexes will be more stable than the complexes formed with monodentate and /or bidentate or tridentate ligands, owing to the chelate effect. EDTA has unpaired electrons on the four oxygen atoms that have single bonds with the carbons and on the two nitrogen atoms (Fujii and Roger, 1978).

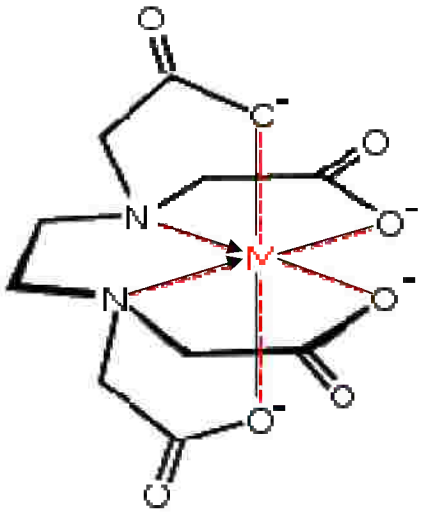

Fig (2): EDTA molecule when fully coordinated to a metal ion, M.

3.3.2.3.1.1. Industrial uses of EDTA

As it is so good at displacing molecules in coordination complexes, EDTA can be used to prevent undesired metals in trace amounts – called impurities - from reacting and having detrimental effects on products. This is known as sequestering. For instance, in regards to cosmetics, EDTA serves to increase the cosmetic product's resistance towards molecules in the air. Simmilarly, in personal care and skin care products, EDTA binds to free metal ions and serves as a purifying agent and perservative. It basically reduces the "hardness" (or presence of metal cations) in tap water so that other ingredients in shampoos and soaps can work to cleanse more efficiently. Along the same lines, EDTA is used in laundry detergents to soften water that comes into contact with it so the other active ingredients can cleanse better. In textiles, EDTA prevents the discoloring of dyed fabrics by removing harmful free metal ions and it also gets rid of residue left on industrial equipment that must be used at high temperatures (ie. broilers). In general, EDTA reduces the reactivity of a metal, preventing any unwanted effects that may result from its presence. EDTA is used in a salt form, most likely in disodium or calcium disodium EDTA. In the chemistry laboratory experiments, students perform one lab of EDTA titrations to determine the % mass of calcium carbonate in an unknown substance (Darwish and Nazek, 1963).

3.3.2.3.1.2. Medicinal and Health-Science Uses

EDTA can also be utilized in medicine. Doctors can prescribe EDTA treatments for patients suffering from lead poisoning. Such a treatment is known as *chelation therapy*, in which EDTA renders the toxic ions present in the body harmless. The EDTA is administered intravenously and makes its way through the blood stream. Given its hexadentate nature, EDTA has a molecular structure much like a claw. Because of this very structure, the EDTA pulls toxic heavy metals detected in the bloodstream towards itself and attaches itself to these metal ions. This attachment forms a compound that can be excreted from the body through urine, not allowing them to bind to enzymes and cytochromes. A chelation therapy may take many sittings and may last anywhere from one to three hours per sitting. Not only can chelation therapy aid in excreting harmful lead ions from the body, but it can also aid in safely getting rid of mercure, chromium, cobalt, nickel, zinc, arsenic and thallium ions from the bloodstream (Oxtoby et al., 2008).

3.3.2.3.2. Cyclam (1, 4, 8, 11-tetraazacyclotetradecane)

Is a tetraazamacrocyclic ligand, which can bind to the central metal ions through the four nitrogen atoms. Thus Cyclam is N_4 chromophoric type ligand. The complexes formed by Cyclam are stable complexes owing to the macrocyclic effect.

3.3.2.3.2.1. Uses of cyclam

The 1, 4, 8, 11-tetraazacyclotetradecane (cyclam) ligand and its derivatives are involved in diverse application fields such as catalysis, enzyme mimics, selective metal recovery, therapy and diagnosis (Meyer, M., et al., 1998). Cyclam and cyclen are the two most important tetraazamacrocycles and their complexes have been widely used as MRI contrasting agents (Bianchi, A., et al., 2000; Merbach, A.E., and Toth, E., 2001), luminescent probes (Reany, O., et al., 2000) , DNA cleavers (Epstein, D.M., et al., 2000) , for labelling antigenic antibodies using radioisotopes (Jurisson, S., et al., 1993; Parker, D., and Chem. Soc, 1990) and medicines for radiopharmaceutical applications (Brechbiel, M.W., et al., 1991).

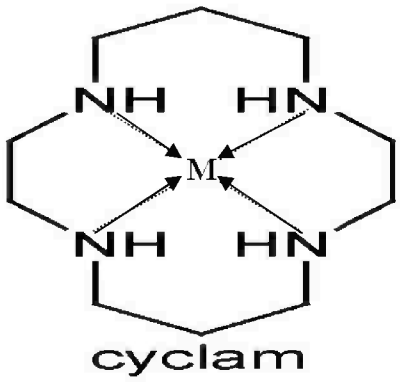

Fig (3) Cyclam molecule when fully coordinated to a metal ion.