

Heavy metal poisoning as a possible cause of massive fish mortality and mongoose in the gold mining area around Khutsong, North west province , South Africa.

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Abstract: Ten composite samples each of water, sediment, fish and organs samples of mongoose from the Wonderfontein area were analysed for the presence of As, Pb, Cd and Cr using atomic absorption spectrophotometry in order to investigate the massive fish mortality in the Wonderfontein stream. Follow up samples of water and sediment were similarly analysed a year later. Abundance of metals followed the trend As>Pb>Cd>Cr, Cr>As>Pb>Cd and As>Cd>Pb>Cr in water, fish and mongoose respectively. The concentrations of As, Pb, Cd and Cr in the initial water samples were 510, 121, 90 and 73 ppm respectively. Water samples collected 12 months later had lower levels of metals at 256, 60, 60, and 50ppm respectively. Cr was highest in fish samples at 56 ppm, while As was highest in mongoose kidney at 25.7ppm. Water concentrations of As, Pb, Cd and Cr were 10 205, 8 020, 2 425 and 733 times higher than the EC/WHO/EPA recommended threshold for potable water while those of Cd and Pb were 36 and 11.2 times higher than those recommended for fish. Levels of metals in sediments were generally higher than those in water. Severe heavy metal pollution with As, Pb, Cd and Cr was revealed in this study, and was also most linked to the mortality of fish and mongoose in the stream. Frequent biomonitoring is therefore recommended in order to safeguard public and animal health in the area.

[Nyirenda M, Thekiso V.V, Dzoma B.M, Motsei L.E, Ndou R.V, Bakunzi F.R. **Heavy metal poisoning as a possible cause of massive fish mortality and mongoose in the gold mining area around Khutsong, North west province, South Africa.** *Life Sci J* 2012;9(3):2533-2537]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 368

Key words: dynamic simulation; model; composting; domestic solid waste

1. Introduction

Contamination of freshwater bodies with a wide range of pollutants has turned out to be a matter of great concern over the past few decades (Vutukuru, 2005; Canli *et al.*, 1998). Among the pollutants are toxic metals, which themselves are natural components of the environment. Anthropogenic activities that include industrial and mining processes however lead to a wider diffusion of these elements in the environment (Miranda *et al.*, 2004). Toxic metals accumulate in water, sediments, soil, plants, and organisms along the food chain (Miranda *et al.*, 2004). Heavy metal contamination may therefore have shocking effects on the ecological balance of the recipient environment (Farombi *et al.*, 2007).

Among animal species, fishes as inhabitants of the more vulnerable aquatic environments, cannot escape the detrimental effects of these pollutants (Olifa *et al.*, 2004). Fishes are therefore commonly used as bio-indicators of aquatic environment pollution in many bio-monitoring schemes (Rashed, 2001; Farkas *et al.*, 2002; Birungi *et al.*, 2007). On the other hand, fishes are an important sources of high-quality protein and beneficial omega-3 polyunsaturated fatty acids (Kucuksezgin *et al.*, 2001).

The study was conducted using samples from the Wonderfontein stream as it passes through Khutsong in

the North west province, South Africa. This was after a mass fish mortality during August, 2009. The headwater of the Wonderfontein originates around the mine residue deposits of several active, old and abandoned mines.

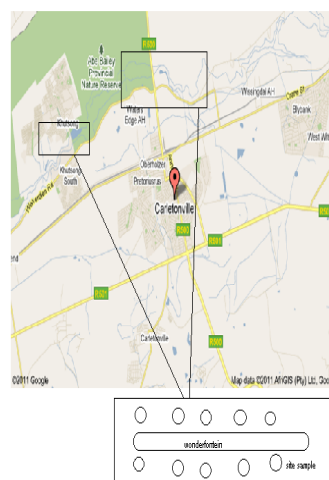


Fig 1: Location map and sampling map of the study area.

2. Materials and Method

2.1 Study area

The study was carried out near Khutsong, a settlement on the outskirts of Carletonville (26°21'S 27°24'E 26.35°S 27.4°E). Carletonville itself is a gold-mining town in western Gauteng, South Africa, and is one of the richest gold-producing areas in the world.

Dam water parameters

The first step in analysing the temporal dynamic of hydrochemical parameters in the Wonderfontein was focused on their variation over the sampling sites. This gives an overview of the range of possible hydrochemical conditions at the time of sample collection. The number of measurements (counts) and selected statistical parameters are compiled in Table 1.

Table 1: Stream water parameters measured in the Wonderfontein in 5 – 10 minutes interval between sampling sites.

Parameter	Temperature	pH
Unit	°C	
Count	5	5
Average	19.1	7.5

Sample collection

Water and sediment

Ten samples each of sediment and water were collected from a 1 km stretch along the stream from the bridge. 500g sediment samples were collected 50cm from the banks of the stream, at a depth of about 10cm. The samples were transported to the laboratory in clean plastic bags. In the laboratory, these samples were combined to make a composite sample that was then well mixed and made into ten 1kg duplicate samples. 500 ml each of water were collected from similar points as sediment, and made into similar composite samples to produce ten 1L duplicate samples.

Fish and mongoose samples

Dead fish and mongoose specimens were collected from the Wonderfontein stream near the Krugersdorp-Carletonville road bridge. These were brought to the laboratory on ice immediately and then frozen at -25 °C until processing for analysis.

Sample preparation

Water

Each water sample was filtered through a 0.45 micron microspore membrane filter in order to avoid clogging of the burner capillary.

Sediment

Sediment samples were put in aluminum plates and left to air dry for 7 days. They were then refined through a 2 mm screen prior to digestion.

Fish and mongoose specimens

About 50g of composite samples was collected from 15 fish, while the same weight was collected from mongoose liver, kidney and muscle tissue. These were dried to constant weight at 85°C in preparation for acid digestion.

Digestion of samples

Equipment preparation

All laboratory equipments used for sample digestion and analysis were soaked in 32% HCl overnight. They were rinsed with distilled water 3 times and dried in a hot air oven for 16 hours at 106° C. A desiccator was used for 6 hours to cool crucibles.

Sediment

Five grams (5g) each of sieved soil and sediments were mixed with 10 ml of distilled water and shaken for 30 minutes. The aqua regia digestion method (Mapanda *et al.*, 2007) was performed for complete dissolution of soil samples. The solution was filtered through Whatman filter paper no 42 into a suitable container. The extracts were used for analysis.

Water

Digestion was performed to ensure the removal of organic impurities from the samples and thus prevent interference (Momodu and Anyakora, 2010). The samples were digested with concentrated nitric acid (where 10ml of nitric acid was added to 50ml of water in a 250ml conical flask). The mixture was evaporated to half its original volume on a hot plate after which it was allowed to cool and then filtered through Whatman filter paper No.42.

Tissue samples

5 ml of HNO₃ and 5ml of H₂SO₄ were added to 5g of tissue samples (dry weight). When the reaction slowed, the tubes were placed in a hotblock digestion apparatus and heated at 60°C for 30 min. After cooling, 10ml of HNO₃ were added, and heated slowly to 120°C, then to 150°C until the samples tubes turned black. After cooling, 1ml of H₂O₂ was added, resulting in a vigorous reaction. Further heating and addition of H₂O₂ were carried out, after which the tubes were removed and filled up to 50ml with distilled water. 40ml of the digest was taken and filled up to 50ml using 5ml of APDC and 5ml of MIBK phase. The samples were shaken vigorously for 5 min after which metals were determined in the MIBK phase (Agemian *et al.*, 1980).

Estimation of heavy metals in acid digested samples

All the acid digested samples water, sediments, fishes and birds were analyzed for Cd, Cu, Pb and Zn using the Atomic Absorption Spectrophotometer (AAS) with the approved methods from the Perkin Elmer

release Version E (2000). Values were expressed as parts per million (ppm).

3. Results

Ten composite samples each of water, sediment, fish and mongoose from the Wonderfontein area were analysed for the presence of As, Pb, Cd and Cr. Follow up samples of water and sediment were similarly analysed a year later. Abundance of metals followed the trend; As>Pb>Cd>Cr, Cr>As>Pb>Cd and As>Cd>Pb>Cr in water, fish and mongoose respectively. The concentrations of As, Pb, Cd and Cr in the initial water samples were 510, 121, 90 and 73 ppm respectively (Table 2). Water samples collected 12 months later had lower levels of metals at 256, 60, 60, and 50ppm respectively. The metals occurred at various levels in fish and mongoose samples, with Cr being highest in composite fish samples at 56 ppm, and lowest in mongoose muscle at 0.22 ppm. On the contrary, as was highest in mongoose kidney at 25.7 ppm. Levels of metals in sediments were generally higher than those in water, except for Cd.

Table 2: Trace heavy metal levels (ppm) in water, sediment, fish, and mongoose specimens from and around the Wonderfontein stream at Carltonville

SAMPLE	ARSENIC	CHROMIUM	CADMIUM	LEAD
WATER 2009	510.25	73.25	90.10	121.25
WATER 2010	305.2	65.61	86.73	65.37
SEDIMENT 2009	581.81	86.01	51.63	943.76
SEDIMENT 2010	348.2	77.07	49.7	812.85
FISH 2009	5.65	56	1.8	2.24
FISH 2010	3.33	54.02	1.63	2.08
MONGOOSE 2009				
Kidney	25.66	0.46	2.65	3.26
Liver	15.16	0.63	6.33	2.33
Muscle	3.25	0.22	0.12	0.65

4. Discussion

The current study revealed massive pollution of the Wonderfontein stream by trace metals As, Pb, Cd and Cr. This pollution can be attributed mining activities in the surrounding gold mines. In the North West Province of South Africa, gold mining activities have been associated with varying levels of heavy metal contamination that has posed potential risks to inhabitants of surrounding informal settlements (Winde, 2002; Winde and van der Walt, 2004; Winde *et al.*, 2004). Biomonitoring of the environment therefore becomes mandatory.

As, Pb, Cd and Cr trace metals in 2009 showed a trend of decreasing in 2010 and this could be attributed to the fact that the mines could have implemented the recommendations of Winde *et al.* report of 2004.

The heavy contamination of fish and mongoose specimens strongly points to heavy metal toxicity as the cause of mortality. A complete post mortem, including histopathology and microbiology were hugely hampered by post mortem time, which led to specimen deterioration for the respective protocols. However, based on the levels of water contamination and tissue levels, chances are high that death was related to environmental pollution. The turbidity of the water at the time of fish deaths further corroborates the findings of high pollution. Exposure of animals to high levels of toxic metals puts them at risk of adverse effects that include reproductive impairment, physiological abnormalities, behavioral modification, or even death (Sarkar *et al.*, 2003, 2008).

The heavy contamination of the specimens also bear testimony to the mobility of heavy metals up the food chain, and the risks it poses to public health. The concentrations of the concerned heavy metals were above the recommended thresholds for water and fish (EC, 2005) (Table 3).

Table 3: Recommended safe metal concentrations (ppm) as stipulated by the EC Regulation (2005)

METAL	Recommended threshold in water	No of Times greater in study water	Recommended threshold in fish	No of Times greater study fish
Cadmium	0.005	18020	0.05	36
Lead	0.05	2425	0.2	11.2
Arsenic	0.05	10205	*2	2.8
Chromium	0.1	733	NA	-

*in meat in general NA- not available

Both sets of water samples, the one at the time of fish deaths and the one 12 months later were huge magnitudes higher than the recommended levels, although the latter ones had lower levels. The finding of excessive contamination of the environment with heavy metals puts the surrounding communities at risk (Winde, 2002; Winde and van der Walt, 2004; Winde *et al.*, 2004), more so because of the fishing, swimming and livestock grazing and watering activities in the area. Arsenic is one of the most toxic and carcinogenic metals derived from the natural environment (Yu *et al.*, 2006). Chronic exposure to inorganic arsenic compounds may result in neurotoxicity of the peripheral and central nervous systems (Goyer and Clarksom, 2001). The main signs of acute toxicity are an acute onset of profuse vomiting, fever, and disturbances of the cardiovascular and central nervous systems which may lead to death (Jarup, 2003). Arsenic has also been associated with cancer in the lung, kidney, bladder, and skin (ATSDR, 2003).

In cattle, clinical signs include depression, prostration, weight loss, weakness, dehydration, anaemia, anorexia, bloody diarrhea, ruminal stasis,

lethargy, dermatosis, reddish urine, dry dull rough, epilated hair coat and anoestrus (Rana *et al.*, 2008).

Pb is associated with a wide range of signs and symptoms that include impaired hearing ability, anaemia, renal failure, reduced immune system, low birth weights, still births and miscarriages, premature births, and elevated blood and urine lead levels in humans (Ati-Hellal *et al.*, 2007; Shahtaheri *et al.*, 2007; Gulser and Erdogan, 2008.).

Cadmium adversely affects a number of organs and tissues such as kidney (induces renal tubular dysfunction, proteinuria and chronic renal insufficiency), heart (aortic and coronary arteriosclerosis, increases cholesterol and free fatty acids), lung (fibrosis), skeletal system, testes, placenta, brain and the central nervous system (Houston, 2007). Its compounds also induce kidney, prostate and testicular tumors in animals, and lung, kidney and prostate cancers in humans (Waalkes, 2003; Satarug *et al.*, 2003). The short-term effects of high Cd concentrations include diarrhoea, nausea, vomiting, renal failure, muscle cramps, salivation, sensory disturbances, convulsions, shock and liver injury (Drastichova, 2004).

Chromium can affect developing fetuses, and can lead to DNA damage, which may result in cancer causing gene mutations (Dingbang *et al.*, 1995). The massive pollution of the Wonderfontein stream exposes the general populace to any of the effects described above.

Conclusion

Heavy pollution of the Wonderfontein stream with As, Pb, Cd and Cr was revealed in this study, which was also linked to the death of fish and mongoose in the stream. More bio-monitoring, public and animal health studies are strongly indicated in this area. Policy regarding point source environmental pollution in the area need to be reviewed and enforced in order to avert a possible future public health crisis.

Acknowledgement:

Authors would like thank the Department of Animal Health, North West University (South Africa) for the financial support to carry out this work.

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References

1. **Vutukuru SS** 2005. Acute effects of Hexavalent chromium on survival, oxygen consumption, haematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *Int. J. Environ. Res. Public Health*, **2(3)**: 456- 462.
2. **Canli M, Ay O, Kalay M** 1998. Levels of heavy metals (Cd, Pb, Cu, Cr and Ni) of *Cuprinus carpio*, *Barbus Capito* and *Choudrostrome* regium from the Seyhan River, Turkey, Tubitak. *Turk J Zool*, **22**: 149-157.
3. **Miranda M, Lo'pez-Alonso M, Castillo C, Herna'ndez J, Benedito JL** 2004. Effects of moderate pollution on toxic and trace metal levels in calves from a polluted area of northern Spain. *Environment International*, **31**: 543– 548.
4. **Farombi EO, Adelowo OA, Ajimoko YR** 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria ogun river. *Int J Environ Res Public Health* **4(2)**: 158-165.
5. **Olifa, F. G., Olifa, A.K. and Onwude, T.E.,** 2004. Lethal and sublethal effects of copper to the African fish (*Clarias gariepnus*). *African Journal of Biomedical Research*, **7**: 65-70.
6. **Rashed MN** 2001. Monitoring of environmental heavy metals in fish from Nasser lake. *Environment International*, **27**: 27–33.
7. **Farkas A, Sala'nki J, Speczia'r A** (2002) Relation between growth and the heavy metal concentration in organs of bream (*Abramis brama L.*) populating Lake Balaton. *Arch Environ Contam Toxicol*, **43**: 236–243.
8. **Birungi, Z., Masola B, Zaranvika MF, Naigaga I, Marshall B** 2007. Active biomonitoring of trace heavy metals using fish (*Oreochromis niloticus*) as bioindicator species. The case of Nakivubo wetland along Lake Victoria. *Phys Chem Earth*, **32 (15-18)**: 1350-1358.
9. **Kucuksezgin F, Altay O, Uluturhan E, Kontas A** 2001. Trace metal and organochlorine residue levels in red mullet (*Mullus barbatus*) from the Eastern Aegean, Turkey. *Water Res*, **35(9)**: 2327–2332.
10. **Mapanda F, Mangwayana EN, Nyamangara J, Giller KE** 2007. Uptake of heavy metals by vegetables irrigated using wastewater and the subsequent risks in Harare, Zimbabwe. *Phys Chem Earth*, **32**: 1399–1405.
11. **Momodu MA, Anyakora CA** 2010. Heavy metal contamination of ground water: the surulere case study research. *J Environ Earth Sci*, **2(1)**: 39-43.
12. **Agemian H, Sturtevant DP, Austen KD** 1980. Simultaneous Acid Extraction of Six Trace Metals from Tissues by Hotblock Digestion

- and Determination by Atomic Absorption Spectrometry. *Analyst*, **105**: 125.
13. **Winde F** 2002. Uranium contamination of alluvial systems- mechanisms and processes. Part I. Geochemical mobility of uranium along the water-path: the Koekemoerspruit (South Africa) as a case study. *Cuadernos de Investigacion Geografica*, **28**: 49- 57.
 14. **Winde F, van der Walt IJ** 2004. The significance of groundwater-stream interactions and fluctuating stream chemistry on waterborne uranium contamination of streams—a case study from a gold mining site in South Africa. *J Hydrol*, **287**: 178–196
 15. **Winde FP, Wade P, van der Walt IJ** 2004. Gold tailings as a source of waterborne uranium contamination of streams - The Koekemoerspruit (Klerksdorp goldfield, South Africa) as a case study Part I of III: Uranium migration along the aqueous pathway. *Water SA*, **30(2)**: 219- 225.
 16. **Sarkar M, Chowdhuri G, Chattopadhyaya A, Biswas NM** 2003. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. *Asian J Androl*, **5**: 27-31.
 17. **Sarkar S, Hazra J, Upadhyay SN, Sing RK, Roy Chowdhury A** 2008 Arsenic induced toxicity on testicular tissue of mice. *Indian J Physiol Pharmacol*, **52**: 84-90.
 18. **EC- European commission Regulation** 2005. Commission Regulation (EC) No: 78/2005 of 16 January 2005 amending Regulation EC No: 466/2001 as regards heavy metals. *Official Journal of the European Union: Legislation Series*, **16(43)**:43-45.
 19. **Yu HS, Liao WT, Chai CY** 2006. Arsenic carcinogenesis in the skin. *J Biomed Sci*, **13**: 657–666.
 20. **Goyer RA, Clarksom WT** 2001. Toxic effects of metals. In: C.D. Klaassen, Editor, *Casarett and Doull's Toxicology. The basic Science of Poisons*, McGraw-Hill, New York, 811–867.
 21. **Jarup L** 2003. Hazards of heavy metals contamination. *Br Med Bull*, **68**: 167-182.
 22. **ATSDR-Agency for Toxic Substances and Disease Registry** 2003. Toxicological Profile for Cadmium, U.S. Department of Health and Humans Services, Public Health Service, *Centres for Diseases Control*, Atlanta, GA.
 23. **Rana T, Sarkar S, Mandal TK, Batabyal S** 2008. Haematobiochemical profiles of affected cattle at arsenic prone zone in Haringhata block of Nadia District of West Bengal in India. *Internet J Hematol*, **4(2)**.
 24. **Ati-Hellal M El, Hellal F, Dachraoui M, Hedhili A** 2007. Plackette-Burman designs in the pretreatment of macroalgae for Pb, Cr and Al determination by GF-AAS. *Comptes Rendus Chimie*, **10**: 839–849.
 25. **Shahtaheri SJ, Khadem M, Golbabaei F, Rahimi-Froushan A, Ganjali MR, Norouzi P** 2007. Solid phase extraction for evaluation of occupational exposure to Pb (II) using XAD- 4 sorbent prior to atomic absorption spectroscopy. *Int J Occup Saf Ergon*, **13**: 137–145.
 26. **Gulser F, Erdogan E** 2008. The effects of heavy metal pollution on enzyme activities and basal soil respiration of roadside soils. *Environ Monit Assess* **145(1-3)**: 127-133.
 27. **Houston MC** 2007. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infraction. *Alternative Therapy in Health and Medicine*, **13**: S128–S133.
 28. **Waalkes MP** 2003. Cadmium carcinogenesis. *Mutat Res*, **533 (1-2)**: 107-120.
 29. **Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PEB, Williams DJ, Moore MR** 2003. A global perspective on cadmium pollution and toxicity in non -occupationally exposed population. *Toxicol Lett*, **137 (1–2)**: 65–83.
 30. **Drastichova J, Svobodova Z, Luskova V, Machova J** 2004 Effect of cadmium on haematological indices of common carp (*Cyprinus carpio* L.). *Bull Environ Contam Toxicol*, **72**:725–732.
 31. **Dingbang C, Jiaming H, Renqiu H** 1995. Whole blood trace element content study of 170 pair neonate and lying-in woman. *Guangdong Trace Elements Science*, **2**:55–9.

9/10/2012