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Preliminary Findings on the Levels of Five Heavy Metals in Water, Sediments, Grass and Various Specimens from Cattle Grazing and Watering in Potentially Heavy Metal Polluted Areas of the North West Province of South Africa

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Abstract: A study was carried out to determine the levels of 5 heavy metal pollutants in water, sediments and grass from the gold mining area of Koekemoerspruit near Orkney in the North West province of South Africa. Fecal and blood samples of cattle grazing and watering from the same area were also analysed. Similar samples from Mafikeng, a mining activity free area 200 km away from Orkney were used for comparison. Determination of heavy metal levels was carried out using the Atomic Absorption Spectrophotometer (AAS) machine, ICP-MS and confirmed on the AAS 700S.Uranium (U), Arsenic (As), Lead (Pb), Cadmium (Cd) and Aluminum (Al) all occurred in varying amounts in samples from both locations. The levels of all the heavy metals analysed for were significantly higher in soil, water and grass samples from Koekemoerspruit than those from Mafikeng, except for Al which was higher in grass samples from Mafikeng. Koekemoerspruit water samples had As, Al and Cd levels of 0.12, 12.8 and 0.01 ppm that were several magnitudes higher than the WHO/EPA maximum permissible levels for drinking water of 0.01, 0.2 and 0.003 ppm, respectively. The animals from both locations had varying levels of all the heavy metals in their serum and faeces. However, significantly higher levels in serum and faeces for Koekemoerspruit were found for U, Cd and Pb, indicating possible implications on the food chain. Sediments had higher levels of heavy metals than water, ranging from 10 times higher for Al to 350 times higher for U. Results of the present survey reveal possible public health risks with regards to the water levels of Al, Cd and As in the mining area of Koekemoerspruit, as well as a generally higher contribution of the mining activities to a higher heavy metal presence in the environment. Owing to the influence of the environmental levels of heavy metals on fecal and serum levels, as well as the excessively high levels of environmental Al, further studies on the possible effects of high environmental levels of the heavy metals on various aspects of animal health and agricultural production are indicated.

Key words: Heavy metals, environmental pollutants, cattle specimens, food chain mobility, agricultural production, influence

INTRODUCTION

Mining of metals, industrial wastes, wastewater and geochemical structure create a potential source of heavy metal pollution in the environment (Karadede and Unlu, 2000; Lee *et al*., 2001; Winde *et al*., 2004; Mapanda *et al*., 2007; Wang and Mulligan, 2009). Under certain environmental conditions, heavy metals may accumulate to toxic concentrations and cause ecological damage (Sivaperumal *et al*., 2007).

The effects on organisms include biological and pathological reactions, reproductive problems and mortality (Janssens *et al*., 2003; Sarkar *et al*., 2008; Praveena *et al*., 2007). 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 Walt, 2004; Winde *et al*., 2004). Various worldwide studies have been carried out on heavy metal contamination of the environment. However, the majority of these studies have been restricted to heavy metal concentrations in water and sediments and studies in fish (Burger *et al*., 2002; Winde, 2002) with little on effects on animal health (Rana *et al*., 2008a, b). The current study therefore seeks to study the influence of mining activities on the levels of

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heavy metals in the environment and in some animal specimens in the gold mining area of Koekemoerspruit in the North West Province of South Africa.

MATERIALS AND METHODS

Study areas: The study was conducted in the Koekemoerspruit area which is near Orkney (26°58'48"S and 26°38'24"E) at an altitude of 1300 m above sea level. Samples were collected from and around the Koekemoerspruit stream that runs through the area. The stream is a tributary of the Vaal river and is a non-perennial stream with a total catchment area of about 860 km^2 (DWAF, 1999; Winde *et al*., 2004). Samples for comparison were collected from Mafikeng (25° 52' 0S and 25° 38' 60E) which has an altitude of 1278 m above sea level and is also in the North West province of South Africa.

Research animals

Animals and experimental design: Twenty adult cattle of both sexes were randomly selected from a communal herd that grazes around and waters from the Koekemoerspruit stream. The stream water derives almost wholly from ground water pumped from the disused Stilfontein gold mine in an effort to prevent flooding in the nearby Buffelsfontein gold mine. Another 20 animals from Mafikeng, grazing on natural veld and watering from a borehole were also randomly selected from a large communal herd and were used for comparison.

Collection of samples: The various samples were collected as detailed below and as shown in Table 1.

Fecal samples: Fecal samples were collected directly from the rectums of the randomly selected cattle using arm length gloves and transported to the laboratory in fecal bottles.

Blood samples: Blood was collected from the jugular veins of the same animals from which fecal samples were collected. Sterile 21 gauge needles were used and the samples were stored in plain tubes that were kept cool in ice cubes during transportation to the laboratory for harvesting of serum.

Sediment and water samples: Twenty each of sediment and water samples were collected from a 1 km stretch of the Koekemoerspruit stream which provided the sole water source for the animals used in the study. Twenty 500 g sediment samples were collected at various distances from the middle of the stream towards the bank, at a depth of about 10 cm. The samples were transported to the laboratory in clean plastic bags. At the laboratory, these samples were combined to make a composite sample that was then well mixed and made into ten 1 kg duplicate samples. About 500 mL each of water were collected from similar points as sediment and made into similar composite samples to produce 10l L duplicate samples.

Table 1: Description of various samples (n = 200) collected for analysis

Sample name	Description	Total
Sediments/soil	20-Mafikeng, soil from the veld 20-Koekemoerspruit stream	40
Grass	20-Mafikeng, natural veld fed upon by cattle in the study 20-Koekemoerspruit, from river bank and veld grazed upon by study animals	40
Serum	20-Mafikeng, study animals 20-Koekemoerspruit area, study animals	40
Faeces	20-Mafikeng, study animals 20-Koekemoerspruit area, study animals	40
Water	20-Mafikeng, borehole water 20-Koekemoerspruit steam water	40

Soil: Twenty 500 g soil samples were collected at 10-15 cm depths from randomly selected points on the natural pasture where the Mafikeng animals were grazing, including around the borehole. The samples were similarly treated as sediment samples.

Grass samples: Grass samples were collected over the same 1 km stretch along the stream, as well as other randomly selected points to represent the grass that the cattle were surviving on. Grass blade samples were collected by randomly picking up about 5 mature bottom blades until a 15 cm bundle was achieved. The samples were transported to the laboratory in khaki bags.

Sample preparation

Fecal and grass samples: Fecal and grass samples were air dried in aluminum plates for 7 days and then ground using a 2 mm screen grinder. Well mixed composite samples each of fecal and grass samples were made and then divided into 10 duplicate samples.

Blood: Blood samples were left for 24 h at a temperature of 4°C to allow clotting and centrifuged at 1000 rpm for 10 min. Serum was then stored in clean plastic tubes and immediately frozen at -18°C until analysis.

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

Sediment and soil samples: 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.

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 h at 106°C. A desiccator was used for 6 h to cool crucibles.

Fecal and grass samples: Dried crucibles with 1 g of sample each were placed in a drying oven for 16 h at 106°C. They were then cooled in a desiccator for 2 h and re-weighed to obtain the dry weight of the sample. The samples were then ashed in the muffle furnace for 16 h at 800°C before being allowed to cool in a desiccator for 4 h. The ash weight of the samples was then determined prior to degstion in preparation for metal analysis.

Sediment and soil samples: About 5 g each of sieved soil and sediments were mixed with 10 mL of distilled water and shaken for 30 min. 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 sample: 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 10 mL of nitric acid was added to 50 mL of water in a 250 mL 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.

Serum: Preparation of serum for metal analysis was performed as per the method of Rana *et al.* (2008a) where a known volume of serum was digested with double the volume of triple acids [Nitric acid 10 parts, Perchloric acid 3 parts and Sulphuric acid 1 part (v/v)] on a hot plate. Dilution to a known concentration was performed with de-ionised water by filtration (Whatman filter paper No. 42).

Estimation of heavy metals in acid digested samplesn: All the acid digested samples of serum, feces, grass, soil, sediments and water were analyzed for U, Pb, Al, As and Cd using the Atomic Absorption Spectrophotometer (AAS) machine, ICP-MS and confirmedon the AAS 700S using approved methods from the Perkin Elmer release Version E (2000).Values expressed as parts per million (ppm).

Data analysis: Statistical analysis of the data was performed using ANOVA following the general linear model of the SPSS program (Version 10.0). The results were expressed as means and pulled SE of Mean (SEM). The means were then compared using Independent t-tests. A significant level of $p<0.05$ was used. Grass metal Concentration Factor (CF) for soil was calculated as Concentration in plant (ppm)/Concentration in soil (ppm) (Mapanda *et al*., 2007) and that for water was also similarly calculated.

RESULTS AND DISCUSSION

A total of 40 samples each of soil or sediment, grass, water, serum and feces from the gold mining area of Koekemoerspruit (N = 20) and Mafikeng (n = 20) were analysed for heavy metals namely uranium, arsenic, lead, cadmium and aluminum.

The levels of all the heavy metals analysed for were significantly higher in sediment or soil, water and grass samples from Koekemoerspruit than those from Mafikeng, except for aluminum which was higher in grass samples from Mafikeng (Table 2). Koekemoerspruit water samples had As, Al and Cd levels of 0.12, 12.8 and 0.01 ppm that were several magnitudes higher than some permissible levels for drinking water.

Animals from both locations had varying levels of the 5 heavy metals in their serum and feces (Table 3). However, significantly higher levels in serum and faeces for Koekemoerspruit were found only for U, Cd and Pb. Serum As and Al levels were significantly higher in Mafikeng samples thanthose of Koekemoerspruit, albeit with significantly lower fecal levels (Table 3). In all the locations, soil or sediments had higher levels of heavy metals than water, ranging from 10 times for Al to 350 times for U. Grasses also had higher heavy metal conversion ratios for water than for sediments or soil (Table 4).

The trace element levels of soil, sediment, water and grass samples are shown in Table 2. All the 5 elements were present in all the samples, with aluminum and uranium showing the highest levels. The earth is known to contain varying levels of heavy metals. U is widely dispersed through out the earth's crust (with an average concentration in rocks and soils of 2-4 ppm) and is usually found in combination with gold where it may form a by-product of gold mining depending on the grade of U involved (Winde, 2002). The 0.077 and 0.00022 ppm in sediment and water, respectively noted in this study therefore suggests low or no environmental pollution in

Table 2: Heavy metal levels (ppm) in sediments or soil (n = 40), water (n = 40) and grass (n = 40) samples from Koekemoerspruit and Mafikeng. Kk = Koekemoerspruit, Mf = Mafikeng, Ss = sediment (Koekemoerspruit) or soil (Mafikeng), Wa = Water, Gr = Grass

	Uranium			Arsenic			Lead			Cadmium			Aluminum		
Location	Ss	Wa	Gr	Ss	Wa	Gr	Ss	Wa	Gr	Ss	Wa	Gr	Ss	Wa	Gr
Kk	0.07700[a]	0.00022000[a]	0.000120[a]	0.238[a]	0.1190[a]	0.00803[a]	1.36544[a]	0.012[a]	0.0085[a]	0.0331[a]	0.0230[a]	0.00219[a]	123.50[a]	12.67080[a]	0.45036[a]
Mf	0.00075[b]	0.00000176[b]	0.000007[b]	0.002[b]	0.0123[b]	0.00388[b]	0.1130[b]	0.033[b]	0.0411[b]	0.0081[b]	0.0054[b]	0.00331[b]	106.74[a]	0.50531[b]	9.34600[b]

[a,b]Means with different letters in a column are significantly different (p<0.05)

Table 3: Levels (ppm) of 5 heavy metals in serum and faeces of cattle grazing around the Koekemoerspruit (n = 40) and Mafikeng (n = 40). Kk = Koekemoerspruit, Mf = Mafikeng, Se = Serum, Fe = Faeces

	Uranium		Arsenic		Lead		Cadmium		Aluminum	
Location	Se	Fe	Se	Fe	Se	Fe	Se	Fe	Se	Fe
KK	0.00220[a]	0.0006300[a]	0.0086[a]	0.0480[a]	0.0042[a]	0.0196[a]	0.00463[a]	0.0034[a]	0.1190[a]	24.314[a]
MF	0.00011[b]	0.0000083[b]	0.0099[b]	0.0048[b]	0.00859[b]	0.0198[b]	0.0021[b]	0.0026[b]	0.3900[b]	14.1138[a]

[a,b]Means with different letters in a column are significantly different (p<0.05)

Table 4: Concentration Factors (CF) of grasses for water and Sediment (SD) for various heavy metals in the Koekemoerspruit

	Uranium		Aluminum		Lead		Cadmium		Arsenic	
Location	SD	Wa	SD	Wa	SD	Wa	SD	Wa	SD	Wa
Kk	1.6×10^{-3}	0.54	0.0036	0.036	0.006	0.71	0.066	0.095	0.033	0.067

the sampled areas. A background U concentration in water of 0.0004 ppm has been noted in water in South Africa while the South African Department of Water Affairs' regulations (DWAF, 1996) stipulate U concentrations below 0.07 ppm and 0.01 ppm for drinking and irrigation water, respectively. The US Environmental Protection Agency's recommended drinking water standard is slightly lower at 0.02 ppm for U while Canadian studies estimated the safe range for drinking water at 0.001-0.06 ppm (Zamora *et al*., 2003). Based on the current results, it can be deduced that no diffuse environmental pollution exists in the sampled areas. However, samples that have previously been taken near tailings deposits of gold mines in the same area have shown high levels of contamination of up to 1000 ppm in water (Winde and Walt, 2004), probably revealing selective pollution in various locations which did not involve the watering points for livestock in this study.

The observation that sediment levels of U were magnitudes higher than those of water was not surprising since it is known that sediments serve as a sink for various anthropogenic pollutants (Davies and Abowei, 2009). High levels of Al at 123 and 106 ppm were found in this study for Koekemoerspruit sediments and Mafikeng soil, respectively.

The current results dwarfs the 0.45 and 0.14 ppm previously documented for soil and stream water (Aloway and Ayres, 1997), probably pointing to potentially high risks of Al toxicities in both sampling sites. The 12.67 ppm Al level in Koekemoerspruit water samples is also way above the 0.2 ppm recommended by WHO/EPA as the maximum permissible for drinking water for humans (Zierold *et al*., 2004) while the 0.5 ppm for Mafikeng was marginally higher.

Al toxicity is regarded as a primary plant growth limiting factor in acid soils while acidification of river systems (owing to acid rain and fertilizers) may lead to toxicant killing of fish (Alva and Summer, 1991). In ponies, Al has been found to decrease the percentage of phosphorus absorbed from the large intestines by 59% such that equines grazing forages that contain elevated concentrations of Al or are contaminated with Al-containing soil may need phosphorus supplementation (Kapusniak *et al*., 1988). Al may also interfere with both intestinal calcium absorption and bone mineralization causing reduced calcium retention and lower bone strength (Zafar *et al*., 2004).

In humans, Al has been associated with impaired cognitive functions (Hewitt *et al*., 1990). The high levels of Al revealed in this study therefore indicates the need for further study into the possibilities of Al toxicity and its potential to negatively affect agricultural production. The 0.12 ppm mean arsenic level for Koekemoerspruit was 12 magnitudes higher than the 0.01 ppm recommended by WHO/EPA in drinking water (Zierold *et al*., 2004) while the Mafikeng levels were below the 0.01 ppm level. This is suggestive of risks of As toxicity to the local Koekemoerspruit population.

The levels of As in serum and fecal samples in this study were however <0.284-0.643 ppm as in clinically affected cattle, respectively (Rana *et al*., 2008c). Heavy metal levels in animal specimens are influenced by environmental levels of the metals (Ona *et al*., 2006). The usual sources of environmental As pollution include mining activities, smelters, fossil fuel power plants and agricultural applications like pesticides, insecticides and cattle and sheep dips (Juhasz *et al*., 2007). Mining activities in the Koekemoerspruit area could have been responsible for the pollution.

The Koekemoerspruit water Cd level of 0.01 ppm was way above the maximum permissible level of 0.003 ppm (Zierold *et al*., 2004) while the Mafikeng level was marginally higher at 0.005 ppm. The Korean Soil Environmental Conservation Act prohibits the use of soils containing over 12 ppm Cd for agricultural purposes (Lee *et al.,* 2001). The maximum permissible Cd level for irrigation purposes is 0.05 ppm (Umali, 1999). The estimated natural concentration of soil Pb ranges from 5-25 ppm (Ona *et al*., 2006). The highest sediment Pb concentration of 1.37 ppm found in this study therefore falls below the stated natural range. However, the Mafikeng water Pb levels were marginally higher than the WHO/EPA maximum permissible level of 0.01 ppm for drinking water while the figure for Koekemoerspruit was similar to the maximum permissible level. In locations that have been deemed polluted with Pb, levels between 100-400 ppm have been noted (Tsadilas, 2000). According to Lee *et al.* (2001), the Korean Soil Environmental Conservation Act requires that soils containing over 400 ppm of Pb need not be used for agricultural purposes. The maximum permissible Pb level for irrigation is 0.5 ppm (Umali, 1999). Heavy metals in plants are a result of their absorption from the soil into roots and other plant parts. Plant uptake of heavy metals varies with soil pH, plant species, type of metal and season among others (Iretskaya and Chien, 1999; Lee *et al*., 2001; Abrahams and Steigmajer, 2003).

The uptake of U depends more on the U content of water than that of soil and could be due to poor desorption of U from soil (Lakshmanan and Venkateswarlu, 1988; Hakonson-Hayes *et al*., 2002). This finding was also evident in the current study where the Concentration Factor (CF) in grass was higher (0.5) for water than for sediments (1.6×10^{-3}). The effect of species on metal uptake could have influenced the higher levels of Al in grass samples from Mafikeng, while the seasonal effects emphasise the importance of sampling over different seasons which however fell out side the scope of this study. Through absorption by plants and possible biomagnification, heavy metals may gain entry into the food chain and become both of agricultural (cattle and crop farming) and public health importance (Winde, 2002; Ghosh *et al*., 2004; Rahman *et al*., 2008). The plant concentration factor (Table 4) is a useful parameter for the assessment of substance migration in the food chain. The CF for all the metals from sediment/soil to grass was similar to that of many vegetables as revealed in literature (Mapanda *et al*., 2007) while the CF for grasses from water was much higher. This may be suggestive of the grasses better efficiency at accumulating metals from water when compared to leafy vegetables, probably putting livestock at higher risk. Grass Pb and Cd levels of 0.0085 and 0.0022 ppm were noted. Pb content in crops grown in uncontaminated areas ranges from 0.1-10 ppm dry weight (Flynn, 1999; Umali, 1999) while leafy vegetables grown in mine dump areas may reach up to 45 ppm (Boon and Soltanpour, 1992).

In general, Cd content in many crops is categorized as beyond the limits at levels >1.5 ppm dry weight (Lokeshwari and Chandrappa, 2006). The above literature figures for various other crops further supports the current findings that the heavy metal levels fell below the critical toxicity levels. Generally, the levels of heavy metals were significantly higher for Koekemoerspruit than for Mafikeng. Environmental levels of most metals are known to be affected by industrial, agricultural and environmental processes that include mining (Karadede and Unlu, 2000; Lee *et al*., 2001; Winde *et al*., 2004; Wang and Mulligan, 2009). Current results are in agreement with these findings, where the higher levels for Koekemoerspruit could be explained by the mining activities in that area. Continual and routine monitoring of environmental pollution levels in such localities is therefore indicated.

The heavy metal contents of serum and fecal samples are shown in Table 3. Heavy metal levels in serum and faeces were generally influenced by environmental levels. As stated earlier, the occurrence of trace metals in grass and animal specimens indicates the upward mobility of heavy metal pollutants through the food chain. Arsenic and uranium have also been detected in cow milk (UNSCEAR, 2000; Rana *et al*., 2008b). Exposure of livestock to high levels of toxic metals engenders adverse effects such as reproductive impairment, physiological abnormalities, behavioral modification or even death (Sarkar *et al*., 2003, 2008). Arsenic is one of the most toxic and carcinogenic metals derived from the natural environment and is a relatively common element that occurs in air, soil and all living tissues (WHO, 2001; Rana *et al*., 2008a, b, c). Exposure to As could be through water, plants and soil (Rahman *et al*., 2008). According to Rana *et al*. (2008c) clinical signs of cattle suffering from As toxicity 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. The researchers further noted that As toxicity results in significant haemoconcentration and that alanine trasferase, aspartate trasferase, blood urea nitrogen and creatinine are sensitive indicators for assessing liver and kidney damage associated with As poisoning. Arsenic compounds have also been found to cause testicular damage and affect spermatogenesis, plasma gonadotrophin and testosterone levels in rats and mice (Sarkar *et al*., 2003, 2008). Further studies are therefore indicated to determine the extent to which environmental pollution could be affecting animal health and reproduction in areas that might be affected. With respect to Pb, there are reports of Pb toxicity to animals, mainly

sheep, through soil and vegetation although, it is stated that the soil-plant transfer of Pb is low at only 1-10% (Alloway, 1990; Abrahams and Steigmajer, 2003; Ona *et al*., 2006). This was supported by current findings of low CF of Pb from sediment to grass.

Abrahams and Steigmajer (2003) further noted that the total daily intake of metals by sheep reflects the degree of soil metal enrichment and is elevated during winter/spring, coinciding with the higher rates of soil ingestion and the generally higher pasture herbage metal concentrations.

Absorption of U from the gastrointestinal tract depends on the solubility of the U compound (Berlin and Rudell, 1986). U transferred to livestock through the ingestion of grass and soil is eliminated quickly through urine and feces, while some appear in milk, thereby making these substances probable specimens for assessing exposure to U (UNSCEAR, 2000). Symptoms of U toxicity in livestock include changes in blood cell morphology, disturbance in thyroid function, increased basal metabolism, changes in hepatic function, hematopoietic deficiency and renal damage and the appearance of albumen in urine (Puls, 1994). Although, naturally occurring U is radioactive, its specific activity is low and carcinogenic effects have not been observed in humans or animals after exposure to naturally occurring U (ATSDR, 1999). Varying degrees of Pb poisoning have been reported in animals reared around different polluted areas (Kottferova and Korenekova, 1995; Ancora *et al*., 2008). Animal studies provide evidence that Cd has developmental effects, such as low fetal weight, skeletal malformations, interference with fetal metabolism and impaired neurological development. Cd can be acquired through inhalation and oral exposure. In animals, chronic inhalation or oral exposure to Cd results in effects on the kidney, liver, lung, bone, immune system, blood and nervous system (Calabrese and Kenyon, 1991; ATSDR, 1999; USDHHS, 1993). That none of the randomly selected cattle sampled in this study showed signs of ill health further supports the assertion of low or no environmental pollution in thesampled areas. However, a comprehensive health check would be required in order to determine the effects of heavy metals on animal health.

CONCLUSION

Results of the present survey suggest that mining activities around Koekemoerspruit have contributed to higher heavy metal presence in the environment, with Koekemoerspruit water samples having As, Al and Cd levels that were several magnitudes higher than the WHO/EPA maximum permissible levels for drinking, indicating possible public health risks. Environmental aluminum levels were particularly way above the expected levels, further pointing to possible risks oftoxicities. Environmental levels of heavy metals had a positive influence on serum and fecal levels.

The positive influence of environmental levels of metals on fecal and serum levels indicates the need for studies on the possible effects of high environmental levels of heavy metals on various aspects of animal health and production while the excessively high levels of aluminum also indicates the need for further study into the possibilities of aluminum toxicity and its potential to negatively affect agricultural production. Also, routine sampling of potentially polluted areas should be encouraged in order keep environmental pollution in check.

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