THE TOXICOLOGICAL STATUS OF VIROTEC'S REAGENTS AND TREATED SOLIDS

TECHNICAL DATA SHEET

VIROTEC

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The purpose of this Technical Data Sheet is to demonstrate that Virotec's reagents are not toxic, dangerous or hazardous, and that contaminated water, soil, rock and tailings treated with Virotec's reagents are generally not toxic to plants, animals or humans.

Virotec's reagents form the core of four waste treatment and environmental remediation technologies: ViroFlow™ Technology, ViroSoil™ Technology, ViroMine™ Technology and ViroSewage™ Technology. In order to establish the potential toxicological properties of Virotec's reagents and treated solids, this Technical Data Sheet will consider three areas of research:

- Potential toxicity of Virotec's "virgin" reagents (i.e., reagent status prior to application);
- Potential toxicity of Virotec's "spent" reagents (i.e., reagent status after use in treatment of waste water); and;
- Potential toxicity of contaminated water, soil, waste rock and mine tailings after treatment with Virotec's reagents.

INTRODUCTION

"Hazardous waste" is a waste with properties that make it dangerous or potentially harmful to human health or the environment. The potential universe of hazardous wastes is large and diverse, with wastes being classified as liquids, solids, gases, or sludges. Hazardous wastes can be the by-product of manufacturing processes or simply discarded commercial products, such as cleaning fluids or pesticides. In the USA, for example, a hazardous waste is categorised as belonging to one of four hazardous wastes lists (i.e., the F-list, K-list, P-list, or U-list) or exhibits at least one of four hazardous characteristics: ignitability, corrosivity, reactivity or toxicity.

Many chemical substances are toxic to plant, microbial, animal and human life. "Toxicity" is a measure of a chemical substance's ability to cause injury to biologic tissue or retardation to or death of plants, animals and humans. Various ways of measuring toxicity have been developed, typically using scales to determine the degree of toxicity posed by a chemical substance, such as the lethal dose or lethal concentration. Among the various chemical substances which may be toxic to animals or humans are a wide spectrum of doses and concentrations.

The Toxicity Characteristic Leaching Procedure (or TCLP) was designed by the United States Environmental Protection Agency (EPA) to determine the mobility of both organic and inorganic analytes present in liquid, solid, and multiphasic wastes. The TCLP test is often used to determine if a waste meets a "toxicity" definition, and was developed to mimic what will happen to a solid when left to age in nature for 20 years under acid leaching conditions.

The test was designed to simulate what will happen to a solid waste under standard landfill conditions. Over time, water and other liquids percolate through landfills. The percolating liquid often reacts with the solid waste in a landfill and may pose public and environmental health risks because of the contaminants it absorbs. The TCLP test determines which of the contaminants identified by the U.S. EPA (and now adopted by other agencies around the world) are present in the leachate from a hazardous waste and accurately identifies what their concentrations are. Therefore, a waste is considered "inert" only if:

- It does not undergo any significant physical, chemical or biological transformation;
- It does not dissolve, burn or otherwise physically or chemically react, biodegrade or adversely affect other matter with which it comes into contact in a way likely to give rise to environmental pollution or harm to human health; and;
- Its total leachability and pollutant content and the eco-toxicity of its leachate are insignificant and, in particular, do not endanger the quality of any surface water or groundwater.

TABLE 1: TCLP DATA FOR VIROTEC'S REAGENT AND THRESHOLD VALUES IN AUSTRALIA AND THE USA FOR CLASSIFICATION AS AN "INERT" SOLID

Contaminant	TCLP Value (mg/L)	Threshold Value (mg/L) NSW EPA	Threshold Value (mg/L) US EPA
Arsenic	<0.01	0.5	5.0
Beryllium	<0.01	0.1	No Limit Set
Cadmium	<0.01	0.1	1.0
Chromium	0.046	0.5	5.0
Copper	<0.01	No Limit Set	No Limit Set
Lead	<0.01	0.5	5.0
Mercury	<0.01	0.02	0.2
Molybdenum	<0.01	0.5	No Limit Set
Nickel	0.018	0.1	No Limit Set
Selenium	<0.04	0.5	1.0
Silver	<0.01	0.5	5.0
Vanadium	0.055	No Limit Set	No Limit Set
Zinc	<0.01	No Limit Set	No Limit Set

Wherever Virotec's reagents have been used to immobilise metal contaminants and neutralise acidity, the spent material has generally been demonstrated by TCLP testing to be non-hazardous (depending on the properties of the initial hazardous waste). An example of results from a TCLP test of a reagent is shown in Table 1.

THE POTENTIAL TOXICITY OF VIROTEC'S "VIRGIN" REAGENTS

> *Background: Eco-toxicity markers and methods*

Each national regulatory jurisdiction has its own criteria of eco-toxicological markers and methods. The US EPA prefers sea creatures, such as minnows and water fleas, which are used to determine the presence of toxic elements in water and soil.

For example, the use of fathead minnows (see Figure 1) in Vitellogenin gene expression in male minnows has been used as a molecular marker of exposure to estrogenic endocrine disrupting chemicals, specifically estradiol and estrone, the synthetic hormone 17-ethynylestradiol, and chemicals that mimic estrogens (e.g., nonylphenols, octylphenols and their ethoxilates) that are present in an aquatic environment (Hutchison, et Al., 2006). Fathead minnows have similarly been used in assessing both the metal toxicity (Hoang, et al., 2004) and ammonia toxicity (Crane, et al., 2005) of water.

Figure 1: Fathead minnow (Pimephales promelas)

The fathead minnow, Pimephales promelas (P. promelas), is a member of the fish family Cyprinidae, the largest family of fish. In the US, fathead minnows originated throughout the Midwest and upper Mississippi River basin, west to Utah, north to Canada, and east to Maine. The fathead minnow is unrivalled in toxicological research which aims to assess the effects of pollution on freshwater. Tolerance of adverse conditions and ease of spawning make the fathead minnow ideal for laboratory culture. Brood stock can be maintained in spawning condition year-round ensuring a constant supply of larval fish for toxicity testing purposes. Adults are omnivorous, eating insects, algae, detritus, and micro-crustaceans. In lakes and deeper streams, fathead minnows are common prey for crappies, rock bass, perch, walleyes, largemouth bass, and northern pike. They also are eaten by snapping turtles, herons, kingfishers, and terns. Eggs of the fathead minnow are eaten by painted turtles and certain large leeches. Although humans do not eat fathead minnows, they are harvested as bait.

Maturity of fathead minnows is reached in four to six months at a size of approximately 40mm for females and 48mm for males. Spawning occurs naturally from May through August in the calm shallows of streams and along shorelines in ponds. Males select and prepare the nest site defending it from intruders and only allow persistent females to enter and spawn. Nests are typically located on the under-surface of submerged stones or branches. Females spawn every two to 16 days producing up to 10,000 eggs in a three-month breeding season. Males protect eggs from predation and cannibalism and fan egg masses with caudal and dorsal fins to increase oxygen availability. Eggs hatch in four to ten days, depending on temperature.

> *Toxicological Analysis of "Virgin" Reagents*

The following analysis of the potential toxicity of Virotec's reagents prior to their application using fathead minnows as an eco-toxicology marker was conducted in 2006. Acute toxicity tests with Pimephales promelas and the water flea, Ceriodaphnia dubia (C. dubia), were conducted according to South Carolina and North Carolina toxicity protocols by an independent laboratory. An acute toxicity test is a method used to determine the concentration of a substance that produces a toxic effect on a specified percentage of test organisms in a short period of time (e.g. 24 hours or 48 hours). In this test, death is used as the measure of toxicity.

Sample preparation consisted of adding 2.0g/L of Virotec's reagent to control water. The mixture was placed on a stir plate for 15 minutes and allowed to settle for one hour. The supernate liquor of this mix was decanted and used as the "100 percent" concentration in the toxicity tests. Organisms were exposed to both control water and the 100 percent concentration.

The South Carolina toxicity test protocol utilized Ceriodaphnia dubia in moderately hard control water (121-180mg/L CaCo3) for 48 hours; the North Carolina toxicity test protocol utilized Pimephales promelas in soft control water (0-60mg/L CaCo3) for 24 hours. Protocols from the "Methods for Measuring the Acute Toxicity of Effluents" and "Receiving Waters to Freshwater and Marine Organisms" were applied, as well as the toxicity testing protocols for South Carolina and North Carolina. All control water results met test acceptability criteria (i.e., >90% survival).

TABLE 2: TEST RESULTS ARE SUMMARIZED BELOW

PASS/FAIL TEST RESULTS			
Toxicity Endpoint	**State Test Protocol**	**Fathead Minnow**	**C. duble LC50**
24 Hr Mortality	***North Carolina***	***Pass***	***NA***
48 Hr Mortality	***South Carolinia***	***NA***	***Pass***

Note: The test is considered a "Pass" if there is no significant difference between control mortality and the test concentration mortality

As shown in Table 2, results indicate that for both the South Carolina and North Carolina test protocols, there was no difference between the survival rate of either fathead minnows or water fleas in the control water or the 100 percent concentration (i.e., survival was >90% for each), and thus no acute toxicity was observed for either species in these tests. It can therefore be concluded that there is no acute toxicity associated with Virotec's reagents.

THE POTENTIAL TOXICITY OF VIROTEC'S "SPENT" REAGENTS

> *Background: Eco-toxicity markers and methods*

Worms have been used as eco-toxicity markers in many parts of the world, including the United Kingdom, Europe and Australia (Morgan, et al., 1988). Acute earthworm toxicity tests have been conducted since the mid-1980s and have been a catalyst for the emergence of earthworms as one of the key organisms in environmental toxicology (Edwards & Bater, 1992), particularly as it relates to contaminated soil and solids.

For example, *Eisenia veneta (E. veneta)* have been used in the United Kingdom to assess the toxicity of Nickel- (Ni) contaminated soil (Scott-Fordsmand, 1998), *Eisenia fetida (E. fetida)*, *Enchytraeus albidus (E. albidus)*, and *Folsomia candida (F. candida)* have been used in Europe to assess the toxicity of chromium III (CrIII) on worms (Lock & Janssen, 2002), and *E. fetida and Lumbricum terrestris (L. terrestris)* have been used in Europe to assess the toxicity of polychlorinated biphenyls (or PCBs) on worms (Fitzpatrick, et al., 1992).

An earthworm's immune system consists of two major components: humoral and cellular. Lysozyme and multi-functional proteins released by leukocytes and chloragogen cells are part of the earthworm's humoral systems; cellular defence mainly involves the activity of free coelomocytcs, macrophages and leukocytes.

Much has been written about the immune system of earthworms with a focus on its humoral and cellular functions. A variety of analyses have sought to develop relevant technologies for looking at how an earthworm's immune system could be used as a marker for environmental pollution.

Research has dealt with the problem of environmental pollution with respect to the animal kingdom, and more broadly in relation to human health. Going further into the inflammatory response and the immune system of earthworms, studies have looked at wound healing, while others have compared cellular and humoral activities under the influence of xenobiotics. There have also been attempts to identify the role of certain cells, particularly those that behave as natural killer cells in their capacity to destroy tumour cell targets.

Figure 2: Earthworms (E. fetida) of this kind are typically used as eco-toxicity markers of contaminated soil and solids

Earthworms are capable of tolerating high concentrations of metals in soils and solids with little effect on physiological functions, and for this reason have been used in eco-toxicological bioaccumulation studies (Marinussen et al., 1997; Langdon et al., 2001; Sandoval et al., 2001). Earthworms are particularly suitable for the assessment of contaminant bioavailability because they are proven metal accumulators, are in full contact with the substrate they consume, and are a key species in the food chain to higher order animals, such as birds, lizards and fish (Paoletti et al., 1998). In the following research, *E. fetida* worms were used as eco-toxicity markers in testing the potential toxicity of "spent" or used metal-laden Virotec reagents.

> *Toxicological Analysis of "Spent" Reagents*

A study was conducted in 2003 to test the biogeochemical stability of a metal-laden Virotec reagent (Maddocks, et al., 2003) by subjecting the reagent to metal bioaccumulation and toxicity tests based on methods developed by the US EPA, European Economic Community and the Organisation for Economic Co-operation and Development (OECD) (OECD, 2000; Sandoval et al., 2001).

The aim of the study was to determine: a) whether the reagent, that had been previously used to treat Acid Mine Drainage (AMD) by removing the metals from a liquid to the solid phase of the reagent, would retain the bound metals as non-bioavailable fractions; and b) the reagent would limit the absorption of heavy metals by E. fetida. The chemical stability of the reagent was assessed by subjecting the "spent" or used reagent solids to a TCLP test.

The reagent was first used to treat AMD by adding the reagent to the contaminated liquid and measuring the total and fractionated volumes of heavy metals in the AMD before and after treatment. Prior to treatment, the AMD contained 198mg/L of aluminium (Al), 7.65 mg/L of cadmium (Cd), 0.87mg/L of chromium (Cr), 118mg/L of copper (Cu), 1.8mg/L of iron (Fe), 1.1mg/L of nickel (Ni), 74.8mg/L of manganese (Mn), and 239mg/L of zinc (Zn).

After treatment, the AMD contained 0.13mg/L of Al, 0.02mg/L of Cd, 0.01mg/L of Cr, 0.04mg/L of Cu, 0.0mg/L of Fe, 0.03mg/L of Ni, 7.97mg/L of Mn, and 0.65mg/L of Zn, clearly indicating that all metals were being concentrated into the matrix of the solid reagent, and that Virotec's reagent was effective in removing heavy metals from AMD.

This finding confirmed other outcomes which showed metal removal rates from AMD and other metal-contaminated water when using Virotec's reagents in excess of 99% for most metals. As a consequence of the treatment, the Virotec reagent contained 42,442mg/kg of Al, 70.9mg/kg of Cd, 17.5mg/kg of Cr, 1,615mg/kg of Cu, 1,005mg/kg of Fe, 9.23mg/kg of Ni, 665mg/kg of Mn, and 2,574mg/kg of Zn, further demonstrating the concentrating effect of the reagent.

E. fetida were then fed a diet of this metal-laden reagent mixed with manure. It has been shown that concentrations of 300mg/kg of Cd; 300mg/kg of Cu; and 1,300mg/kg of Zn induce death in *E. fetida*. However, it was hypothesised in this study that, because Virotec's reagents are not toxic after use, feeding worms this potent brew of heavy metals would result in only a limited bioaccumulation of heavy metals in the worms (Cd, Cu and Zn were measured) due to the sequestering effect of metals in the reagent, and would not result in worm weight loss or increased mortality.

Six different colonies of *E. fetida* were fed: 1) 0% reagent + 100% cow manure; 2) 10% reagent + 90% cow manure; 3) 20% reagent + 80% cow manure; 4) 40% reagent + 60% cow manure; 5) 60% reagent + 40% cow manure; and 6) 80% reagent + 20% cow manure. The weight of the *E. fetida* in each colony was measured before the study and after 28 days.

Results showed that worms in all diet categories retained their average weight (approximately 3.0g each) after feeding on the metal-laden reagent, and only accumulated 2.3mg/kg of Cd, 18.66mg/kg of Cu, and 34.98mg/kg of Zn. The study also showed that none of the diet categories resulted in increased worm mortality, indicating that the metal-laden Virotec reagent was not toxic to worms.

Assessment of the data using standard bioaccumulation factors therefore showed that a Virotec reagent "loaded" with high concentrations of heavy metals does not pose an eco-toxicological threat to soil biota in either acute or chronic toxicological aspects. This finding indicates that most metals bound by Virotec's reagents are retained in such a way that they cannot easily be removed from the reagent matrix, will not readily bioaccumulate in soil biota, and therefore will not be returned to the ecosystem.

A second aspect of the study compared the heavy metal soil concentrations of various natural and industrial habitats with the bioaccumulation of metals by worms inhabiting these settings. For example, it was shown that the level of Cd in soil surrounding a zinc smelter is 20mg/kg, but up to 200mg/kg of Cd is accumulated by worms (Ma, et al., 1983), a 900% increase in concentration.

Similarly, it has been shown that soil surrounding a sewage treatment plant contains 9.2mg/kg of Cd, but more than 47mg/kg of this dangerous metal is accumulated in *E. fetida* worms (Hartenstein, et al., 1980), a 400% increase in concentration.

However, it was shown in this study when inhabiting solids composed of Virotec's reagents where heavy metals were high (41.8mg/kg of Cd, 598mg/kg of Cu, 59mg/kg of lead (Pb), and 1,695mg/kg of Zn), *E. fetida* worms only accumulated 2.3mg/kg of Cd, 18.6mg/kg of Cu, 0.24mg/kg of Pb, and 35mg/kg of Zn, representing a lower concentration in the worms (95% for Cd, 97% for Cu, 99% for Pb, and 98% for Zn) than in the surrounding solids. These data indicate that the accumulation of metals in worms was actually less in the worms after they were fed metal-laden reagents for 28 days, further confirming the non-bioavailable and non-toxic nature of Virotec's reagents.

Results of this study therefore demonstrate that Virotec's reagents are effective in binding metals from aqueous solutions, such as AMD, and removal rates are generally >99% for most metals. The reagent can be considered as a potential source of environmental toxicity due to the total concentration of metals bound within it, but this study showed that the reagent did not cause acute toxicity in earthworms and retained biogeochemical inertness under natural environmental conditions.

THE POTENTIAL TOXICITY OF CONTAMINATED SOLIDS AFTER TREATMENT WITH VIROTEC'S REAGENTS

> *Background: Eco-toxicity markers and methods*

The presence of a contaminant, such as a heavy metal like lead, in a soil or solid is sometimes a poor indicator of availability and potential toxicity to plants, animals and humans, because the mere presence of a contaminant does not mean it is "bioavailable".

For example, the presence of organic matter is critical in the control of heavy metal behaviour in soils, and many of the organic constituents in soils have the propensity to form chelates with heavy metals, which has considerable environmental importance as the retention of metals may limit their availability to biological targets within the ecosystem.

In Australia and New Zealand, molluscs, bivalves, prawns (also called "shrimp") and other shellfish have been routinely used as markers of eco-toxicity due to their acute sensitivities to heavy metals and other contaminants, such as fluoride (e.g., Black & Bott, 2005). For example, prawns have been used to assess nitrofuran toxicity (Food Standards Australia New Zealand, 2005), dioxin toxicity (Food Standards Australia New Zealand, 2006), and metals toxicity (McPhee, 2001).

Contamination with heavy metals is a particular risk with many current industrial and agricultural practices. If contaminated solids or soils are allowed to enter a water body by wind drift or erosion by stormwater runoff, they create a potential source of toxic contamination on the seafloor or in lakes, dams or ponds.

Subsequently, toxic materials may leach into the pore spaces within the underlying sediments, adversely affecting animal life that burrow and feed in the sediment layers (so-called infauna) through bioaccumulation of metals and poisoning.

Toxic effects may pass up the food chain when other animals, such as crabs, prawns and fish, consume local infauna. Contaminants incapable of biodegradation may persist in local ecosystems for an extended period of time, being recycled through the food chain long after animals that have

Figure 3: Fully grown tiger prawns typical of the kind used as eco-toxicity markers of contaminated soil and water in aquaculture

accumulated contaminated materials die and provide nourishment for detritus feeders and scavengers. Similarly, toxic materials may be passed through the food chain to animals from adjacent ecosystems that forage in or seek prey from the contaminated area.

In addition, water-soluble contaminants and contaminants adsorbed to suspended particulate material may be carried beyond the area of local contamination. Such transported material may be absorbed across gill surfaces of fish and accumulated in the tissues of exposed aquatic animal life or absorbed by aquatic plant life. Suspended particulate material may also be ingested by filter-feeders, such as oysters and mussels.

Toxic effects associated with the absorption of heavy metals and other contaminants cover a wide range of potential problems depending on the duration of exposure, dose ingested or absorbed, and the sensitivity of the exposed organisms.

Generally, higher exposures and doses cause death, whereas lower concentrations affect animal mobility and behaviour. These chronic effects may lead to starvation due to a diminished capacity to capture prey or forage, or an inability to evade predators. Other chronic effects include inhibited growth or development and reduced fertility and breeding success. These latter effects may be very subtle and only manifest over an extended period. A decline in populations of affected organisms may significantly alter the balance and structure of marine ecosystems.

> *Toxicological Analysis of Contaminated Soil Treated with Virotec's Reagents*

In 2003, an assessment of Acid Sulphate Soils (ASS) contaminated with aluminium (Al) and iron (Fe) was conducted at a prawn farm in south-east Queensland, Australia (Fergusson, 2003). The Tomei Australia prawn farm is located 30 kilometres south of Brisbane and its ponds were excavated in lowland mangrove flats on the foreshore of Moreton Bay. Because the blood composition of tiger prawns (Penaeus monodon), for example, is copper based, prawns are particularly susceptible to the presence of Al and Fe in soil and water.

Tomei Australia had previously identified an ASS problem in a number of their ponds which had detrimental effects on water quality (e.g., high toxic metal concentrations and low pH) significantly affecting prawn mortality and therefore farm production and viability. Among other things, ASS was releasing significant quantities of sulfuric acid, Al and Fe into the bottom of the growing ponds, all of which are highly toxic to post-larvae prawns (PLPs) during their initial growing stages (from 5mm to 25mm), and ASS was binding phosphates and other nutrients needed for the growth of natural food.

As a result, overall prawn yields were extremely low in the affected ponds. For this reason, Virotec was contracted to apply its ViroSoil™ Technology, using ViroBind™ reagent, to solve the problem.

Figure 4: Post-larvae tiger prawns prior to stocking the treated ponds

After the application of ViroSoil™ Technology, Penaeus monodon samples were taken from Ponds 7 and 12, along with their adjacent Ponds 8 and 11 which acted as controls. As shown in Figure 5, toxicological analyses performed by Australian Laboratory Services indicated there was insignificant metal uptake in prawns in the treated ponds, with results being equal to (for Al) or lower than (for Fe) those levels found in control Ponds 8 and 11.

From this data it can be concluded that contaminated soil, having been treated with Virotec's ViroBind™ reagent, does not allow the bioaccumulation of metals in prawns, is not toxic to prawns, and hence toxic metals cannot migrate up the food chain to human consumers.

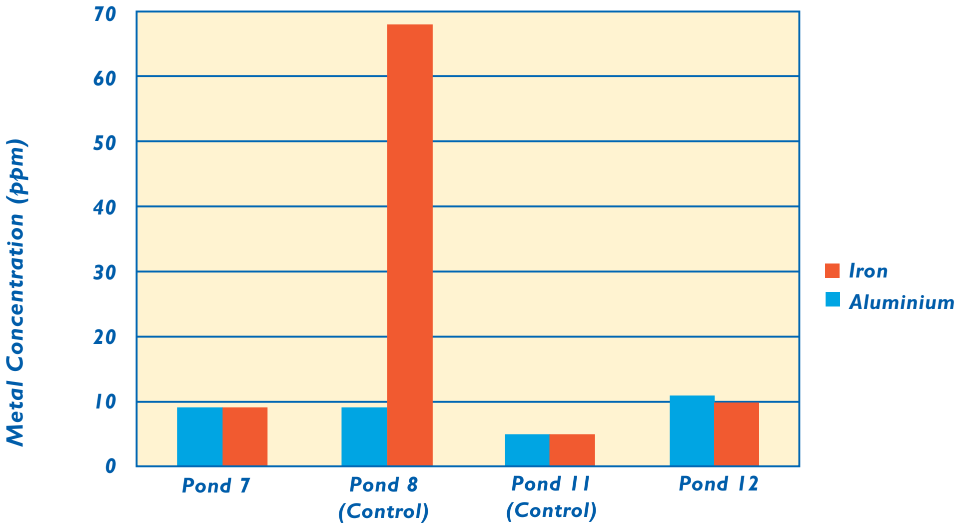

Figure 5: Metal concentrations in tiger prawns from treated Ponds 7 and 12 and from control Ponds 8 and 11

However, it is important to note that soil pH, sand and water metals concentrations, and toxicology are relevant only if pond yields increase, because the only relevant figure for a commercial prawn farm is pond yields. These are measured as kg of prawns per hectare (kg/ha) of pond. After harvest, prawn yields in Pond 7 were found to have increased from 50kg/ha in 2002 to 2.7tonnes/ha in 2003, and from 30kg/ha to 4.3 tonnes/ha in Pond 12. Figure 6 presents the historical and post-ViroSoil™ Technology prawn yields for Ponds 7 and 12.

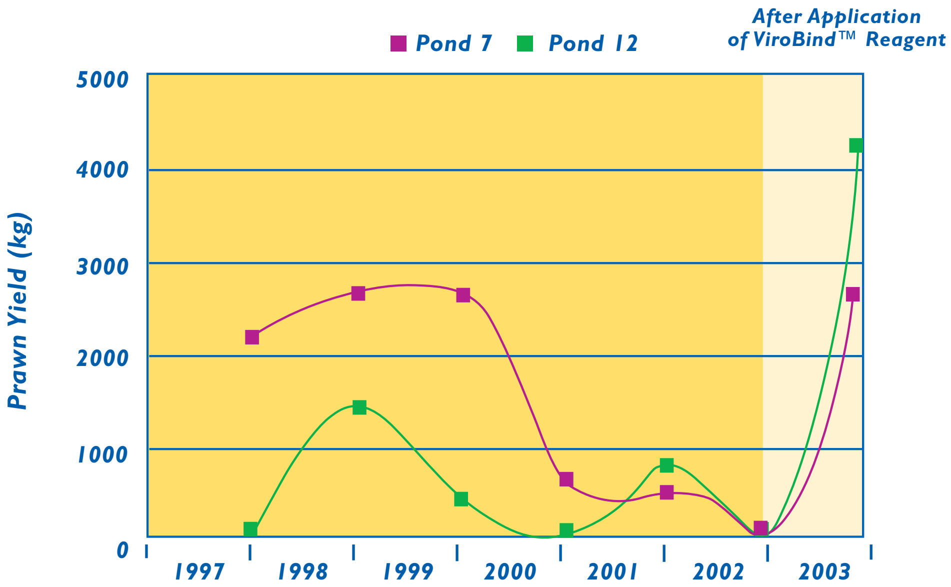

Figure 6: Prawn yields for Ponds 7 and 12 from 1997 to 2003

As a result, the ponds experienced a PLP survival rate that was significantly higher than previously observed in these ASS-contaminated ponds. Moreover, the treated ponds demonstrated healthy algal blooms, and were successful in providing a stable and non-toxic food source for PLPs

In summary, Virotec's reagent when applied to Tomei Australia proved to be successful in treating the ASS problem by lowering Fe and Al concentrations in the soil and water. In addition, toxicology analyses conducted by a NATA-accredited, independent laboratory confirmed that no increase in metals uptake in prawns had occurred and, as a result, prawn mortality (and hence pond yields) increased dramatically. It can also be concluded that there was no threat of metal contamination in humans when consuming prawns produced in ponds which have been treated with ViroSoil™ Technology.

The application of ViroSoil™ Technology at Tomei Australia proved to be extremely successful by improving pond yield, water quality, and the profitability of this prawn farm. As a result of these findings, application of the Technology has continued in other ponds at Tomei Australia for many years.

> *Toxicological Analysis of Contaminated Mine Water, Waste Rock and Tailings Treated with Virotec's Reagents*

Study of the toxicological effects of pollution on plants typically focuses on air pollution. However, increasing attention has been turned to the affects of contaminated soil and other solids on plant, animal and human life. For example, research has been conducted on the long-term effects of pesticides, insecticides and other agricultural chemicals on plant life (Bell & Duke, 2005). Certain species of plants are good bioaccumulators of heavy metals, and the science of phytoremediation explores the use of such plants to treat contaminated water or soil. However, where a plant (wheat or maize for example) forms part of a more complex food chain, the bioaccumulation of heavy metals is undesirable.

Indeed, not only is it undesirable from an ecological sustainability point of view, but the presence of heavy metals themselves is potentially dangerous to the life of the plant, not just problematic for the life or well-being of the birds, animals and potentially humans further up the chain which feed on it. In these situations, the presence of heavy metals and high acidity levels in soil may be enough to inhibit the growth or even kill plants growing in such contaminated soil.

Mine sites are one industrial example of how the presence of high heavy metal concentrations and acidity in soil and rock can inhibit or completely nullify the ability of plant to grow. The problems associated with remediating and revegetating waste rock and tailings on mine sites are well documented and include metal toxicity, inherent acidity, high salt concentrations, poor nutrient content and poor physical structure as the main inhibitors (Kabata Pendias & Pendias, 1992; Alloway, 1995; Dollhopf, 1998; Miekle et al., 1999; Brown et al., 2000). Acidic waste rock and tailings also disallow natural fungal and bacterial ammonification processes and present an ongoing obstacle to plant growth and natural regeneration (Bengson & Thompson, 1998), in some cases doing so for many years (or decades) until oxidation of the material ceases, acid generation is halted and the material is fully weathered and neutralised.

Virotec has analysed the effect of treated heavy-metal contaminated mine waste on plant growth and mortality. The treatment effects of ViroMine™ Technology on plant and animal toxicology were assessed at a derelict mine site in New South Wales, Australia. At the mine, contaminated tailings dam water, contaminated tailings, and contaminated waste rock have all been the subject of toxicological analysis. The Mt Carrington mine carries a legacy of more than 150 years of gold and silver mining (some copper, zinc and antimony were also recovered), with commercial operations ceasing in 1989.

Mineralogical recovery of gold and silver employed the carbon-in-pulp cyanide extraction method. After extraction of gold and silver the tailings were discharged at a pH of >9.0 to a 14 ha tailings dam. After 1989, the water in the tailings dam progressively became acidic and was enriched with heavy metals, largely as a result of the input of AMD water from oxidising waste rock, and in 2001 the tailings dam threatened to overflow because the increasing volume of water could not be treated using existing technology.

The first stage of mine treatment using ViroMine™ Technology involved the treatment of low pH contaminated waste water in the tailings dam with the reagent Acid B Extra™. The quality of the dam water before and after treatment is shown in Table 3.

TABLE 3: WATER QUALITY IN THE TAILINGS DAM

Parameter	Before Treatment with Acid B Extra™ Reagent	After Treatment with Acid B Extra™ Reagent
pH	3.8	7.3
Cadmium (mg/L)	0.4	<0.001
Copper (mg/L)	6.8	0.15
Iron (mg/L)	18.7	3.2
Lead (mg/L)	4.2	0.026
Zinc (mg/L)	42.2	2.1

Due to the increase of pH and reduction of heavy metals, after successful treatment with the reagent, the majority of dam water was released and the remainder was used as a lake for Australian native fish (silver perch, Bidyanus bidyanus) and other aquatic life, such as tadpoles (see Figures 7 and 8).

Figure 7: The silver perch in this photo are being used as toxicity indicators in mine water treated with ViroMine™ Technology

As documented earlier in the Technical Data Sheet, in this application heavy metals were concentrated in the reagent and tailings at the bottom of the dam. As a result, the bottom of the dam was "coated" with a blanket of Acid B Extra™ reagent, and has been shown to be non-toxic to fish, tadpoles and other aquatic life seven years after the initial application. Analysis has shown that this treated sediment is "non-hazardous", according to New South Wales EPA guidelines.

Figure 8: This photo shows a healthy tadpole swimming on the bottom of a tailings dam after treatment with ViroMine™ Technology

In addition to the finding that the treated dam water was not toxic to aquatic life, the revegetation of the tailings dam and surroundings also became possible. The high levels of heavy metals contained both within the treated tailings and in the metal-laden reagent were not toxic to plant life. Moreover, the treated "wet" or submerged tailings were revegetated with aquatic reed species, and the surrounding "dry" or exposed tailings were revegetated with grasses, shrubs and trees, as shown in Figure 9.

Figure 9: After treatment with ViroMine™ Technology, this photo shows healthy reed growth in treated AMD dam water (centre) and healthy grass and tree growth in treated mine tailings (foreground)

Waste rock constitutes the largest volume of material that must be stabilised and remediated at mine sites and is often the hardest to remediate because of their chemical and physical properties.

The waste rock can have both immediate (actual) contamination problems and future (potential) contamination problems that will develop as sulphide minerals continue to oxidise; both the actual and the potential problems must be addressed as part of any long-term solution. The leaching of AMD from waste rock dumps can lead to adverse environmental effects, including contaminated waterways and adjoining terrestrial habitats.

Prior to treatment with Virotec's Terra B reagent, the waste rock had the levels of acidity and heavy metals shown in Table 4.

TABLE 4: WASTE ROCK PROPERTIES PRIOR TO TREATMENT

Parameter	Waste Rock Prior to Treatment with Terra B™ Reagent
Waste Rock pH	4.2
Leachate pH	4.2
Aluminium (mg/kg)	5,575
Cadmium (mg/kg)	14
Chromium (mg/kg)	24
Copper (mg/kg)	420
Iron (mg/kg)	24,756
Lead (mg/kg)	596
Manganese (mg/kg)	827
Nickel (mg/kg)	19
Zinc (mg/kg)	795

Grass and trees generally do not grow on contaminated waste rock due to the low pH (typically grass and trees will not grow in a pH less than 5.5) and high metals content of the exposed and oxidised rock. However, as shown in Figure 10, waste rock, when remediated with Virotec's Terra B™ reagent, will readily grow grass. This demonstrates the non-toxic nature of waste rock once treated with Virotec's reagents.

Figure 10: This photo shows the effect of revegetating waste rock with native grasses after treatment with ViroMine™ Technology (centre) in relation to untreated contaminated waste rock (foreground)

Furthermore, data taken from the three waste rock dumps (one control, one treated with lime, and one treated with Terra B™) showed the treated waste rock dump had an average leachate pH of 7.2 four years after treatment, compared to standard lime treatment which resulted in a leachate pH of 4.8 after four years and unchanged for the control.

Similarly, the heavy metal content of the leachate from the treated dump showed Al at 0.0mg/L, Cd at 0.3mg/L, Cu at 0.01mg/L, and Pb at 0.01mg/L, with most at close to zero for most years and remaining stable after four years. Again by way of comparison, lime treatment resulted in metal levels in the order of 5.0mg/L for Al, 1.5mg/L for Cd, 2.5mg/L for Cu, and 0.25mg/L for Pb. Control leachate levels were significantly higher at 35mg/L for Al, 5.0mg/L for Cd, 10.0mg/L for Cu, and 0.2 mg/L for Pb.

Figure 11: This photo shows the effect of attempting to revegetate untreated waste rock with native trees

Both the treated and untreated waste rock dumps were also planted with a range of equally numbered and healthy native tree species. As shown in Figure 11, the unremediated waste rock dump could not support tree growth at all well. This photograph was taken four years after trees were planted, and shows only a few of the trees survived, with an average tree height after four years of <200mm.

Figure 12: This photo shows the effect of revegetating waste rock with native trees after treatment with ViroMine™ Technology

However, as shown in Figure 12, the treated waste rock was not toxic to trees, and supported their growth. The average height of trees on this waste rock dump was 2.25m after four years, in comparison to 1.0m for the lime dump. These findings confirm that waste rock treated with Virotec's reagents are not toxic to grass and trees.

CLASSIFICATION OF REAGENTS

Virotec's reagents are used to treat a variety of industrial and municipal applications, including industrial waste water treatment, contaminated soil and site remediation treatment, municipal effluent and biosolids treatment, mine site remediation, and other applications. The reagents used in Virotec's technologies have several remarkable characteristics that make them ideal for environmental remediation of land and the treatment of water. They have an excellent phosphorus binding capacity, a significant acid neutralising capacity, and an excellent heavy and trace metal binding capacity.

Moreover, metals that are bound when the reagents are used to treat contaminated water or solids are held very tightly and cannot be easily re-released and liberated to the environment. The typical chemical composition of "virgin" reagents is as follows:

Hematite	***33%***
Beohmite	***18%***
Water	***17%***
Gibbsite	***10%***
Quartz	***8.0%***
Cancrinite	***6.0%***
Sodalite	***4.0%***
Anatase	***2.0%***
Other minor minerals	***2.0%***

"Other minor minerals" include anhydrite, bassanite, diaspore, euxinite, fluorite, gypsum, halite, lepidocrocite, monazite, portlandite, whewellite, and zircon. The mineral constituents of Virotec's reagents are not known to be toxic to humans or animals either individually or collectively. Wherever Virotec's reagents have been applied to immobilise heavy metal contaminants, the spent reagents have generally been demonstrated by TCLP to be inert. Virgin or spent reagents may also be used as inert or immobilised solids in landfill or as a surface additive to regenerate and improve the agricultural value of contaminated land.

> *Hazardous and Dangerous Waste*

In addition to the assessment of toxicity of reagents, the broader question of the potential hazardous and/or dangerous nature of the reagents is relevant. Virotec's reagents have therefore also been considered in the light of a number of regulatory jurisdictions, and the following summary is provided in relation to Australian regulations by way of example.

Environment Australia administers the "Hazardous Wastes (Regulation of Exports and Imports) Act 1989", Australia's obligation under the "Basel Convention on the Control of Trans-boundary Movements of Hazardous Wastes and their Disposal". The guidelines to the Act define waste in terms of its proposed end use, as do similar guidelines for the regulations of wastes by various State authorities. Under these guidelines, where treatment of a waste leads to its transformation into a usable state, the modified waste (for example, contaminated soil after treatment with ViroSoil™ Technology) is not characterised as a waste and is therefore not subject to the Hazardous Wastes Act.

Similarly, certified testing has been performed on Virotec's reagents to demonstrate that they do not meet the criteria for either Class 8 or Class 9 of the Dangerous Goods Code. Virotec's reagents are therefore not subject in Australia to the provisions of either the Hazardous Wastes Act or the Dangerous Goods Code.

In addition, it has been shown that the longer the residue or sediment from Virotec's reagents is left to age in the environment after use, the more tightly the metals are held, and as the residue ages new metal trapping capacity develops.

For example, if the residue is left in a tailings dam or pit-lake after the completion of mine site treatment, metal concentrations in the water will continue to decrease for at least three years and less metal will be released from the sediment after that time than when the application initially took place. It is this capability of Virotec's reagents to continue binding metals long after application that enables them to be used to convert and re-classify hazardous solid waste into a non-hazardous, inert form.

CONCLUSIONS

This Technical Data Sheet provides clear evidence that Virotec's reagents are not toxic before or after use, and shows that contaminated solids (such as soil, tailings and waste rock) treated with Virotec's reagents is not toxic. Results of acute toxicity tests with fathead minnow conducted by independent laboratories under South Carolina toxicity protocols and with water fleas under North Carolina toxicity protocols proved that there is no acute toxicity associated with Virotec's "virgin" reagents. Similarly, TCLP tests conducted on the virgin reagents show that they are non-toxic when compared to the NSW and US EPA guidelines for a hazardous waste.

TCLP tests used to access the chemical stability of metal-laden reagents demonstrated that the "spent" material is also classified as an inert, non-toxic substance. A study of earthworms which had consumed metal-laden Virotec reagents using standard bioaccumulation factors showed that the reagent when "loaded" with high concentrations of heavy metals does not pose an eco-toxicological threat to soil biota in either acute or chronic toxicological aspects. This result indicates that Virotec's reagents are effective in binding metals from aqueous solutions, such as AMD, with heavy metals removal rates in excess of 99%.

Virotec's reagents have also been used in aquaculture production where ASS poses both an environmental and economic threat. Virotec's reagents successfully treated the ASS problem by lowering iron and aluminium concentrations, and by buffering soil pH. In addition, Virotec's reagents improved prawn yield, water quality and profitability of the prawn farm operation. Most importantly, toxicological analysis conducted by an independent, NATA-accredited laboratory confirmed that there was no significant increase in metals uptake in prawns when compared to controls, and therefore no reportable toxicity to eco-toxicological markers was reported.

Virotec's reagents have been used to treat contaminated mine site dam water, toxic mine tailings, and waste rock. Results of this research has shown that the treated water, the dam sediment, treated tailings and waste rock were not toxic to plants or animals up to four years after treatment. Given that standard tests of toxicity use event horizons of 20 years, it can be concluded that Virotec's reagents and solids treated with the reagents remain chemically non-toxic for at least two decades, and this event horizon moves further out into the future as repeat test are conducted.

Moreover, Virotec's reagents, both prior to and after application, cannot be classified as either a hazardous or dangerous waste, as defined by regulatory guidelines worldwide, including the Hazardous Waste Act. For these reasons, Virotec's reagents are considered safe to transport, safe to handle, and safe to apply, and are not toxic to plants, soil biota, fish or other aquatic life when assessed using worldwide standards of toxicological practice. Virotec's reagents are therefore considered to be fully sustainable and healthy for the environment.

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