



## The effect of water pollution on biological control of water hyacinth



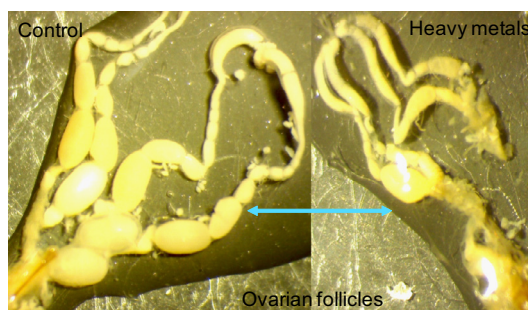
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### HIGHLIGHTS

- We tested the effect of heavy metals and acid mine drainage (AMD) on the biocontrol agent *Neochetina* Spp.
- Copper and arsenic reduced *Neochetina* feeding and ovarian development.
- AMD with sulphates  $\geq 700$  mg/L reduced ovarian development and larval feeding.
- Water pollution from mining in South Africa could constrain biocontrol of water hyacinth.

### GRAPHICAL ABSTRACT



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### ABSTRACT

South Africa has one of the world's biggest gold mining regions with an associated problem of acid mine drainage (AMD), which increases the bioavailability of heavy metal contaminants in water. The prevalence of water hyacinth (*Eichhornia crassipes*) in South African water systems, despite the release of seven biocontrol agents since 1974, is often attributed to high levels of eutrophication. Metal concentration in plant shoots is known to affect insect herbivory. Nevertheless, little is known about the effect of heavy metals or AMD on *Neochetina eichhorniae* and *Neochetina bruchi*, which are the most widely established biocontrol agents on *E. crassipes* in South Africa. Herein, the effect of eight different heavy metals common in AMD (arsenic (As), gold (Au), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), uranium (U) and zinc (Zn)), as well as three different simulated AMD concentration treatments (low, medium and high), on the performance of *Neochetina* weevils were investigated by releasing adults on plants growing in tubs and pools, three weeks after the addition of individual metal or AMD treatments. After six weeks, the number of weevil larvae per plant, the number of adult survivors per plant, the number of adult feeding scars on leaves, and the number of larval mines per plant were recorded. Two females of *N. eichhorniae* and *N. bruchi* from each tub were dissected and the number of ovariole follicles was counted. Adult feeding in *Neochetina* was significantly reduced on plants exposed to both Cu and As while larval feeding was significantly reduced on plants exposed to Hg, Zn, As and Cu, with Cu causing the greatest effect. Similarly, weevil feeding and reproduction were reduced in the medium and high concentration AMD treatments. Larval development was significantly impaired by both metal and AMD treatments. These negative effects disagree with published data which showed no effect of metals on *Neochetina* weevils. The disparity is explained by long exposure of the weevils on whole plants, rather than short exposure to excised leaves, as recorded in the literature. Finally these findings provide evidence that some heavy metals and AMD might be constraining biocontrol agents of water hyacinth in South Africa.

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## 1. Introduction

Acid mine drainage (AMD), which increases the bioavailability of heavy metal contaminants in water and compromises water qualities, is a serious problem in South Africa which is one of the biggest gold mining regions in the world (Cukrowska et al., 2010). The effect of AMD on biological control agents of water hyacinth has not been studied before. However, research has shown that metals in plants can affect insect herbivory (Davis et al., 2001; Coleman et al., 2005; Boyd, 2010).

### 1.1. The effect of heavy metal on herbivorous insects

There are over 450 plant species of hyperaccumulators of both trace metals and metalloids (As, Zn, Ni, Mn, Cu, Co, and Cd), and non-metals such as Se and Ni, and the majority of them occur in metalliferous soils (Verbruggen et al., 2009). Most aquatic macrophytes are also capable of accumulating large amounts of heavy metals in their tissues. Among these are water hyacinth (Malik, 2007; Liao and Chang, 2004; Misbahuddin and Fariduddin, 2002), duck weed, *Lemna gibba* L. (Vaillant et al., 2004), and *Lemna minor* (Alvarado et al., 2008) water fern, *Azolla caroliniana* (Bennicelli et al., 2004), parrot's feather (*Myriophyllum aquaticum*), creeping primrose (*Ludwigia palustris*), and water mint (*Mentha aquatica*) (Kamal et al., 2004). *L. gibba* L. has been indicated as a hyperaccumulator of heavy metals in several studies (Kara et al., 2003; Vaillant et al., 2004; Mokhtar et al., 2011). High levels of metal sequestration in plant tissues protects plants from natural enemies (Boyd, 2010), and this is suggested to compromise the management of invasive alien weeds using biological control agents, which are specialist herbivores. For instance an increased Cd concentration in alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb) reduced the fecundity of the alligatorweed flea beetle *Agasicles hygrophyla* Selman and Vogt up to 92% (Quimby et al., 1979). Copper concentrations between 0.01 and 0.64 mg/L Cu in water reduced first-instar feeding of *Paratanytarsus parthenogeneticus* Freeman (Diptera: Chironomidae) on green algae (Hatakeyama and Yasuno, 1981).

Evidence for heavy metal or AMD effects on *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) is limited and largely based on Cd. Even so, the results of weevil interaction with such metals are inconsistent and contradictory. For instance, feeding damage caused by the weevil *N. bruchi* was significantly reduced when the biocontrol agent was exposed to accumulated concentrations of 232 µg Zn/100 g d. wt., and 66.70 µg Cd/100 g d. wt. in water hyacinth (Jamil et al., 1989a,b). In contrast Hussain and Jamil (1992) found no mortality or any other symptoms in adult *N. eichhorniae* feeding on plants grown in water with solutions of Cd, Zn, Hg, and Mn at concentrations up to 100 mg/L. Similarly, Kay and Haller (1986) found no significant feeding damage or mortality of *N. eichhorniae* fed on water hyacinth exposed to concentrations of 1–5 mg Pb/L, 0.5–1.0 mg Cd/L or 1–2.5 mg Cu/L for ten days.

### 1.2. Feeding and reproduction of *Neochetina* weevils

The water hyacinth weevils, *N. eichhorniae* and *N. bruchi*, are the most widely used biocontrol agents of water hyacinth in South Africa. Both weevils can cause considerable damage to water hyacinth but have only satisfactorily controlled the plant at one site in South Africa (New Years Dam in the Eastern Cape) (Byrne et al., 2010).

The nocturnal adults of both species are about 4–5 mm long and spend the day sheltering in the leaf sheath or inside rolled leaves (DeLoach and Cordo, 1976; Oberholzer, 2001). On average each

female produces 350–400 eggs which are laid either within young leaf tissues by *N. eichhorniae* and on the upper surface of older petioles by *N. bruchi* (Oberholzer, 2001). There are three larval instars (DeLoach and Cordo, 1976). Njoka (2004) found that the egg duration of *N. bruchi* and *N. eichhorniae* were 11 and 13 days, respectively, while those of the larvae were 31 and 55 days, respectively to complete their developmental stages and the pupae took 31 and 25 days, respectively, until eclosion. On hatching, the neonate larvae start to tunnel into the petiole and burrow towards the crown of the plant. The adult weevils feed on the epidermal layer of the leaves, usually leaving behind characteristic feeding scars on the upper and lower leaf surfaces and, less so, on the petioles (Del Fosse et al., 1976; DeLoach and Cordo, 1976).

Rates of larval and adult feeding and egg production in both *Neochetina* species depend on temperature and nutritional quality of the plants (Grodowitz et al., 1997). Under unfavourable conditions (e.g. poor nutrient quality of host plant), egg production degenerates at the expense of development of flight muscles, a process that is reversed when suitable conditions are encountered (Buckingham and Passoa, 1985; Grodowitz et al., 1997).

In South Africa reproduction and population expansion of the weevils on water hyacinth starts in spring, when the mean daily temperature rises above 20 °C and the plants start to grow vigorously. However, the weed remains a problem, reportedly because many South African water bodies are excessively and consistently enriched with nutrients (Coetzee et al., 2011), allowing water hyacinth to compensate for the weevil damage. Low winter temperatures (below 10 °C) in some parts of South Africa also curb the weevil populations, allowing the water hyacinth populations to resurge while the weevil populations lag behind (Hill and Olckers, 2001). Reproduction and feeding of the *Neochetina* species could be reduced by accumulations of heavy metals in their host plants caused by AMD increasing the bioavailability of the metals in water. In this study the effect of eight different heavy metals and different concentrations of AMD on a mixed population of the *Neochetina* weevils via their host plant were investigated.

## 2. Materials and methods

The effects of heavy metals and AMD on *Neochetina* weevils were investigated as single heavy metal tub trials and a suite of heavy metal treatments mixed artificially with different concentrations of sulphates in a simulated “AMD” pool trial. Because the uptake of heavy metals by plants is affected by several environmental factors, including ionic competition (Prasad et al., 2001; Tangahu et al., 2011; Deng et al., 2004), the two trials allowed comparison of the weevils' feeding and reproduction under conditions where there was little or no ionic competition for uptake by plants (the tub trial) and under conditions where there was complex interaction between the different ions in water (the AMD trial).

The adults of both *Neochetina* species that were used in each of the trials were reared at the South African Sugarcane Research Institute (SASRI) in Kwazulu Natal province. Three female weevils from the single-element tub trial and 12 from the pool trial were randomly taken from the mixed population of both the *N. eichhorniae* and the *N. bruchi*, and dissected to determine the functional status of the ovaries (parous and nulliparous) before the start of both trials. The experimental set up of both the single-metal tub and simulated AMD pool trials was the same, as those shown in Newete et al. (2014).

### 2.1. Experimental set up

#### 2.1.1. Single-element system tub trial

Three replicates of 13 treatments with a total of 39 plastic tubs of 65 L were arranged randomly in four rows under a clear non-UV

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